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FULL PAPER

# Change in body weight of mothers and neonates and in milk composition during denning period in captive Japanese black bears (*Ursus thibetanus japonicus*)

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## Abstract

**Japanese black bears, *Ursus thibetanus japonicus,* have been classified as a vulnerable species so that data on reproduction are needed to maintain and/or extend their population. They are known to have a peculiar style of reproduction, giving birth to their neonates and raising them during denning, a period of complete fasting. In this study, we investigated the metabolic rate and milk composition of mother bears raising neonates, and the changes in body weight of the neonates under captive conditions. Seven female bears kept in dens were weighed once a month, and the amount of energy they used was calculated. From birth, cubs were also weighed and their growth rate was determined. In addition, the milk composition was analyzed to investigate its characteristics. As a result, it was found that mother bears used 34% more energy than did solitary females. There was no significant difference in the energy used for nursing whether they had single or twin cubs. On the other hand, the body weight gain of single cubs was significantly higher than that of twin cubs, suggesting that the growth of the cubs was highly affected by the suppression of mother's energy consumption during the fasting period. The milk had high fat and low sugar concentrations. This indicates that mother bears used the fat accumulated prior to denning for their main energy source when raising cubs. Considering all results together, Japanese black bears showed remarkable efficiency in the use of energy for reproduction during the fasting period.**

Key words: Japanese black bear, neonatal growth, milk composition, denning

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## Introduction

Generally, black bears and brown bears are known to give birth during the denning period[2,16]. The denning of bears is different from the hibernation of small mammals in many respects. Two of the most significant differences are that bears neither eat nor drink for about 4–5 months from late November or early December[23] and that neonates are born in the middle of this period[2,16,33]. It is also notable that the neonates are more immature at birth and that the proportion of neonatal body weight to the maternal body weight is much smaller in comparison with that of other carnivores, because the net gestation period lasts only for about two months due to a unique reproductive mechanism in bears called delayed implantation[27,32]. After parturition, mother bears keep feeding their neonates milk until they are mature enough to emerge from the dens in spring. The milk composition is the crucial factor for the life and growth of neonates. Under a fasting condition, however, milk production also requires enormous energy from mothers. Thus, female bears have to store energy before hibernation not only to maintain themselves, but also for the growth of the fetus, parturition, and lactation. Successful reproduction must be supported by the physiological features, including milk composition and metabolic rates, which are adapted to the fasting condition of mother bears. From this perspective, Ursidae is the only genus among terrestrial mammals which adapt to a fasting condition with parturition and nurturing.

The Japanese black bear (*Ursus thibetanus japonicus*) is a subspecies of the Asiatic black bear that lives on Honshu and Shikoku Islands, Japan. Today, their population is reported to be gradually decreasing, and four local groups in the western part and one in the northern part of Japan are categorized as "Threatened Local Populations" in the Red Data Book published by Japanese Ministry of the Environment[22], since large numbers of bears are killed, because of being regarded as a nuisance by humans. It will soon be necessary for us to make efforts for their *ex-situ* conservation, and now it is important to accumulate fundamental physiological knowledge of the reproduction and neonatal growth of Japanese black bears.

The unique characteristics of bear reproduction are great concerns not only for wildlife management but for research into their outstanding physiological mechanisms. However, it is difficult to investigate these phenomena during hibernation, especially in free-ranging wild bears. To date, milk components have been investigated in some bear species such as American black bears[27], brown bears[3], polar bears and sun bears[18], but serial data are limited. The growth rate of neonates has been reported in the Hokkaido brown bear[33], grizzly bear[10], American black bear[10,27], and giant panda[28]. However, there have not been any reports yet on neonatal growth and milk composition in Asiatic black bears including Japanese black bears. In this study, we investigated the metabolic rate and milk composition of mother bears raising neonates, and the changes in body weight of their neonates under captive conditions during the denning period, aiming to reveal the reproductive features of this species.

## Materials and Methods

*Animals:* Four captive, sexually mature female Japanese black bears (3 pregnant and 1 non-pregnant) were kept in dens during the '96–'97 winter season, as well as five pregnant bears during the '97–'98 winter season at Ani Mataginosato Bear Park, Akita, in northeastern Japan (40°N, 140.1°E). The same two bears were kept in dens in both seasons so that a total of 7 bears were used throughout the 2-year experiments. All bears were fed cornmeal and commercial bear pellets during the active season (from mid-April to mid-December) and were moved indoors for denning on December 20–25 in 1996, and on December 20 in 1997. During the denning season, bears had free access to water but not food. Seven of the 8 pregnant bears gave birth to their cubs during the

**Table 1. Seven bears used in this study and their reproduction profiles**

| No. | Pregnancy | Date of Parturition | Litter size | Sex of Cubs |
|---|---|---|---|---|
| 31 | + | Jan. 26, 1997 | 1 | F |
| | + | Jan. 25, 1998 | 2 | M, F |
| 32 | + | Jan. 30, 1997 | 1 | M |
| | + | Jan. 23, 1998 | 2 | M, F |
| 37 | + | Feb. 3, 1997 | 1 | F |
| 38 | − | − | | |
| 42 | + | − | | |
| 46 | + | Jan. 30, 1998 | 2 | M, F |
| 49 | + | Feb. 7, 1998 | 1 | M |

denning period (Table 1).

*Handling and measurement of body weight (BW):* Blood and milk sampling, and body weight (BW) measurement were performed in January, March, April, and December in 1997, and once a month between January to April in 1998. The bears were anesthetized by blow dart injections with either of the combinations of the following drugs: zolazepam HCl and tiletamine HCl cocktail (Zoletil®, Virbac, Carros, France) 5.0 mg/kg, or xylazine HCl (Celactar®, Bayer, Germany) 1.0 mg/kg and ketamin HCl (Ketaral®, Sankyo, Japan) 5.0 mg/kg. BW was measured with a spring scale, and blood samples were taken from the jugular vein after immobilization. In 1998, milk samples were collected from four nursing bears with oxytocin 100 IU intramuscular injection for assistance, and stored at −20°C for analysis. The BW of each cub was measured using a bucket placed on a scale.

*Milk composition:* Milk samples obtained from the four nursing bears were analyzed. Analysis methods for each component (milk solids, fat, protein, sugar, sialic acid and ash) are described below.

a ) Solid content: Five hundred mg of each milk sample was freeze dried. Then, the solid matter was weighed in order to determine the solid content/100 g of milk.

b ) Fat: Fat content was quantitatively determined by the Rose-Gottlieb method[14], with modification for making the scale smaller.

c ) Protein: Non-Interfering Protein Assay (Geno-Technology, Inc., St. Louis, Mo, USA) was performed. A standard curve was prepared with casein (Merck, Darmstadt, Germany).

d ) Sugar: The phenol sulfuric acid method[9] was used.

e ) Total sialic acid: Sialic acid content was quantitatively determined by the metaperiodate resorcinol reaction[19]. Oxidation of metaperiodate was performed at 0°C.

f ) Ash: Five hundred mg of each milk sample was desiccated over 100°C, and then burned in an electric furnace at 550°C for about 5 hr until all milk changed into a light-colored ash. Ash content/100 mg of milk was weighed.

*Statistics:* Statistical analyses of the serial change of milk composition were conducted using GraphPad Prism (GraphPad Software Inc., San Diego, CA), by repeated one-way analysis of variance, followed by the Tukey test for *post hoc* testing. To determine significant differences in BW change between the two bear groups, each slope over time was compared. Comparisons were considered significant at $P < 0.05$.

## Results

*Change in body weight (BW) and metabolic rate (MR) of mother bears during winter:* Relative change in BW (kg) of mother bears with BW = 100% at parturition as the criterion is shown in Fig. 1. BW at parturition was calculated as follows. All parturitions occurred between the 23rd of January and the 7th of February. Daily weight decrease between the January and February measurements was calculated by formula 1 below. In this formula, $DAY_{Jan\text{-}Feb}$ represents the number of days between the January and February measurements.

Formula 1:
Daily weight decrease between January and February = $(BW_{Feb}(kg) - BW_{Jan}(kg))/DAY_{Jan\text{-}Feb}$

Then, BW of female bears at the time of parturition was calculated by formula 2.

Formula 2:
$BW_{parturition} = BW_{Jan} -$ Daily weight decrease between January and February $\times DAY_{Jan\text{-}parturition}$.

BWs of mother bears were constantly decreased during denning (Fig. 1). The heaviest mother bear was 77.7 kg and the lightest was 53.5 kg at parturition from calculation by formula 2. Applied to a linear regression model, the regressed BW ratio of mother bears with a single cub and twin cubs after parturition was expressed as follows: BW ratio = $-0.0022x + 1.00$ (x: days after parturition, determination coefficient: 0.8451) and BW ratio = $-0.0025x + 1.00$ (determination coefficient: 0.8713), respectively. There was no significant difference in the BW decrease ratios between mother bears with a single cub and those with twin cubs after parturition ($P > 0.1$).

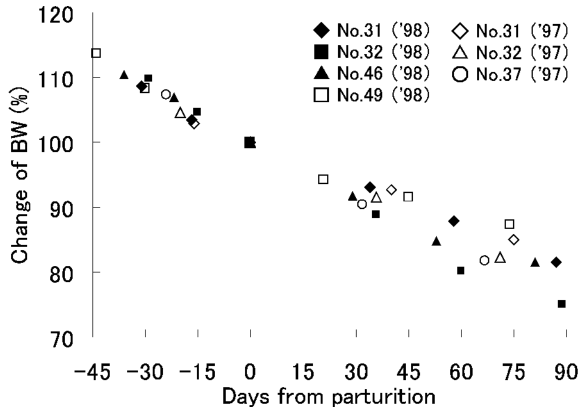


**Fig. 1. Change in body weights (BWs) of 5 female denning black bears with single and twin cubs before and after parturition.** BWs constantly decreased during the experimental period. Bears No. 31 (◆), No. 32 (■) and No. 46 (▲) gave birth to twin cubs in 1998, and No. 31 (◇), No. 32 (△), No. 37 (○) in 1997 and No. 49 (□) in 1998 had only one cub (open objects). There was no significant difference in decreased BW ratio between females with single and twin cubs after parturition ($P > 0.1$).

The MR of each bear per day was calculated by formula 3 below (Farely and Robbins, 1995) (Table 2). In this formula, BWD means the total body weight decrease during the entire experimental period.

Formula 3: MR (kcal/day) = BWD (g) × Fat calo-

**Table 2. Metabolic rate of seven female bears during denning in 2 experimental years**

| Year | Bear No. | Neonates | Metabolic rate (kcal/day) |
|---|---|---|---|
| 1997 | 31 | + | 1,400 |
| | 32 | + | 1,200 |
| | 37 | + | 1,500 |
| | 38 | − | 900 |
| 1998 | 31 | + | 1,606 |
| | 32 | + | 1,529 |
| | 42 | − | 1,224 |
| | 46 | + | 1,300 |
| | 49 | + | 1,453 |

rific value (9.1 kcal/g)/total days

The average metabolic rate of pregnant and nursing bears was 1,426.89 ± 52.75 kcal/day, which is 34% higher than the 1,061.77 kcal/day of bears without cubs. In bears No. 31 and No. 32, the metabolic rate tended to be higher in 1998 (1,606 and 1,530 kcal / day, respectively), when they had two neonates, than in 1997 with one neonate (1,400 and 1,200 kcal / day). However, the metabolic rates between bears with one cub and those with two cubs were not significantly different (Fig. 1, Table 2).

*Growth rate of bear cubs:* The monthly change of bear cub BWs is shown in Fig. 2. After the first measurement in February, their BW increased at an almost constant rate. Applied to the linear regression model, the BWs of a single cub and twin cubs were expressed as follows: BW (kg) = 0.0327x + 0.1593 (x; days from birth) and BW (kg) = 0.0198x + 0.2382, respectively. The determination coefficient was 0.8725 for single cubs and 0.9603 for twin cubs. There was a significant difference in the growth rate between the two groups ($P < 0.005$).

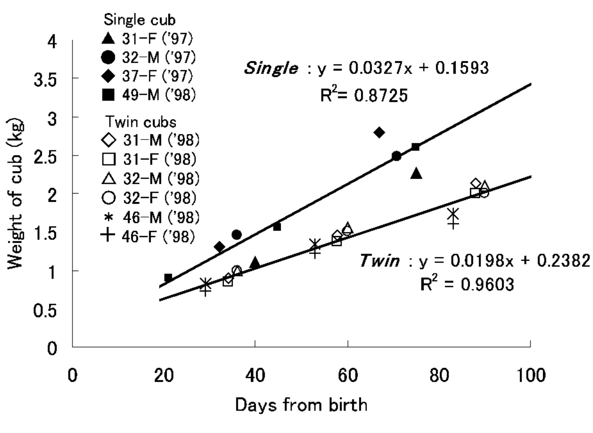


**Fig. 2. Body weights (BWs) of cubs measured by scale once a month from February to April.** Graph legend represents the tag No. of the mother bear, sex and birth year of the cub. The cubs of No. 31, No. 32, No. 37 female bear in 1997 and No. 49 in 1998 were single cubs (closed symbols), and the others were twins. Each equation of the regression line for the single cub group or twin cub group was determined by the linear regression model. There was a significant difference in the growth rate between the two groups ($P < 0.05$).

*Milk composition:* Milk in the amount of 2.5-6.0 ml was obtained by hand milking from every pregnant bear in 1998. We also obtained milk from bear No. 42 that did not deliver cubs. Milk from this bear presented a yellow color that differed from the white-colored milk from nursing bears. This milk was not used for analysis of milk composition.

The average values of solid content, protein, fat, sugar, ash and total sialic acid in milk from nursing bears (n = 4) from February to April were 27.22%, 6.88%, 16.22%, 2.37%, 1.32%, and 0.70%, respectively. Variations of milk composition of nursing bears are shown in Fig. 3. The fat and sugar concentration of nursing bears' milk increased significantly from February (11.60% and 1.56%) to April (21.11% and 3.16%), respectively.

## Discussion

In the present study, we first investigated change in the BW of mothers and neonatal bears during denning, and the characteristics of milk composition in Japanese black bears. Bears were provided enough food in the active period, and then moved indoors for denning in late December without food. Although most wild Japanese black bears enter their dens in late November to early December in Japan[13,20], some bears were reported to start denning in late December[20]. Thus, we consider the artificial influence in these experiments could be mostly neglected. Denning bears are in a state of prolonged fasting, and female bears must use the energy and nutrients for pregnancy, parturition and lactation from their body reserves accumulated prior to denning. According to a previous study which demonstrated that bears use fat as the main source of energy during denning[23], we calculated the metabolic rate of female bears by multiplying the body weight decrease by the heat capacity of fat (9.1 kcal/g) as per formula 3. As a

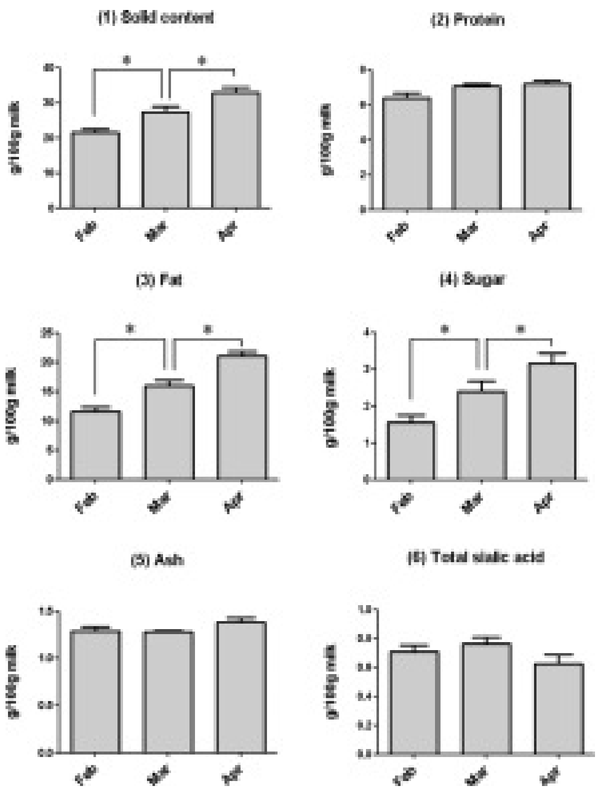


**Fig.3. Milk composition determined with milk samples from 4 nursing bears taken from February to April.** Each figure expressed the concentration (%) of (1) solid content, (2) protein, (3) fat, (4) sugar, (5) ash and (6) total sialic acid. Vertical bars represent + SE. Concentration of solid content, fat and sugar significantly increased while they were nursing cubs during denning ($P < 0.05$).

result, nursing bears used approximately 34% more energy than non-nursing bears. Farely and Robbins[10] reported that the cost of lactation for nursing two cubs would increase the total over-winter loss by 23% in female American black bears and 47% in female grizzly bears, compared with those with no cubs. This additional energy was supposed to utilize mainly for the development of their neonates and milk production. In the present study, however, the average metabolic rate was estimated to be 1,426.89 ± 52.75 kcal/day for nursing bears, a value as low as the basal metabolic rate of a 65-kg man[6] (Table 2). It was suggested that bears suppressed their energy consumption as much as possible during winter denning.

As shown in Fig. 1, the BW decrease of females with single cubs and those with twin cubs were not significantly different, which suggests that there was not much difference in the total maternal investment for a single cub and twin cubs. In other words, mother bears nurse their cubs within certain investment limits whether they have one or two cubs. This could be the means not only for raising cubs but also the maintenance of their own lives given their limited resources during the prolonged fasting period. On the other hand, it is reported that the more cubs females raised in the denning period, the greater the over-winter loss of BW becomes in American black bears, if they had two to four cubs[30]. Thus, in the case of three cubs or more, the BW loss of female bears might be much greater. In our results, the same bear having nursed neonates in both experimental years also tended to consume a larger amount of calories when it had twin cubs, so that a greater sample is needed to reach a conclusion. If female bears give birth to more than three cubs, much more energy should also be needed in Japanese black bears. However, in this study, the litter size was one or two for all female bears that bore cubs. Two cubs are reported to be the majority in other reports on the litter size of Ursidae, in Hokkaido brown bears[33], giant panda[28], American black bears in Florida[12] and polar bears[7].

In contrast to the non-significant difference in the BW of mother bears by litter size, the growth rates of neonates showed a significant difference between a single cub and twin cubs. Figure 2 shows that the daily BW gain of single cubs (32.7 g/day) is approximately 1.65 times greater than that of twin cubs (19.8 g/day). Thus, a single cub could obtain more energy from its mother than twin cubs, and this result coincides with the data described above that show the amount of BW loss was not different between mother bears with single and twin cubs. For reference, the daily BW gain of single cubs of Japanese black bears was less than the 49 g/day of twin cubs in American black bears and 98 g/day of twin cubs in grizzly bears[10]. These differences may correspond with their differences of adult body size. For example, adult male Japanese black bears (50–120 kg)[5] are smaller than adult male American black bears (60–300 kg), and far smaller than adult male brown bears (135–390 kg) (http://www.bearbiology.com/).

The BW of neonates at birth was estimated to be 159 g for the single cub group, and 238 g for the twin cub group by the linear regression model. But these BW calculations could be underestimated. The BW of Japanese black bears at birth was measured to average about 300 g in the neonates of this bear park (unpublished data). The development of bears from birth to one month may be more gradual. Bears are very small at birth as reported by many researchers, and the baby represents only 0.3–0.5% of maternal BW compared with 1–5% in most carnivores[27]. This small baby size is essential for bears that nurse their cubs during the fasting period to reduce glucose consumption and the use of body fat for the energy of neonatal growth because embryo and fetus growth mainly needs glucose[4,11]. During fasting, body protein is mainly used for gluconeogenesis and it becomes impossible to recover from a collapse of protein homeostasis in a state of overly prolonged fasting. Bears must minimize the need for gluconeogenesis despite transferring their energy to the young. This is thought to be a reason why female bears deliver smaller neonates in mid-winter[29].

The milk composition of mother bears also showed the characteristic of suppressing glucose consumption. Influence of using oxytocin on the bear milk composition has not been investigated so far. However, intramuscular oxytocin injection had been taken by many researches for determining bear milk composition[10,18,27], and composition of the milk of the brown bear, *Ursus arctos*, taken in the same period was reported to be similar whether oxytocin was used or not[3,10]. So we think oxytocin injection had insignificant influence on the values of milk composition in this study. In the present study, we clarified that their milk contains low sugar, high fat and high protein compared with other terrestrial mammals. In Japanese black bear milk, the sugar concentration was 1.6% in February and increased to 2.8% in April. A similar tendency was reported in American black bears by Farely and Robbins[10], and these values were lower than the milk of other terrestrial mammals such as ruminants (4–5%), horse (6–7%), human (6–7%), pig (3–5%), dog (3%) and cat (4–5%)[15,17], reflecting the need to reduce glucose consumption of mother bears. Interestingly, the dominant carbohydrates in the milk of bears are known to be oligosaccharides[34–36], whereas disaccharide lactose is a major component in the milk of most other eutherian species. The milk oligosaccharides are now thought to be significant as anti-microbials in the young[21], but it is not known whether bear cubs can digest and absorb them.

The fat concentration in milk is usually less than 5% in mammals such as humans, ruminants and horses[15,17,25]. Milk of carnivores like dogs and cats has comparatively high fat concentration, approximate 7–12%[1,15,25]. Compared with the milk fat concentration of these animals, that of Japanese black bears in the present study (16.22%) is much higher. The high ratio of fat calories in the total milk calories in Japanese black bears suggest that most of the energy used for neonatal growth is obtained from fat, which is also the energy source of mother bear survival during hibernation. Among bear species, polar bear milk is reported to contain the highest fat concentration (33.1%). For reference, aquatic mammals such as seals, whales and sea lions produce milk containing much more fat (45–61%) than bears[8,26]. These aquatic mammals also nurse their young during periods of no, or less, food. On the other hand, reindeer also secrete milk that contains about 18% fat, which thought to be related with their migration. Comprehensively, milk with a high fat concentration seems to be an effective adaptation for ecology of each mammal. In bear species, fasting could be the most critical factor for milk composition. In this study, fat concentration increased significantly from February to April. These findings are in agreement with earlier studies in other bear species such as American black bears, Hokkaido brown bears and Grizzly bears[3,10,18,27]. During denning, cub body mass increased more than about 5 times even in twins in this study (Fig. 2), so that nutritional demands for cub growth could be also increasing. These demands could be partly covered by increase of milk intake by cubs, as reported in grizzly bear and American black bear[10]. Also, as shown in the present study, increasing concentration of highly-demanded nutrients like fat and sugar in bear milk was likely to be important, because the high solid content, which means little water was contained in the milk, could be effective to avoid dehydration during period without drinking water. We think this is a reason why fat and sugar content in bear milk increased during denning period. As to other components, the increase in solid content during hibernation mainly reflects the increase of fat. Changes in protein, total sialic acid and ash content were not observed. From these results, the milk of Japanese black bears apparently has the common features of bear milk, such as high fat and low carbohydrate levels, and these two components increase along with neonatal growth during denning.

The results of the present study can be summarized as follows: In Japanese black bears, the mother bears used 34% more energy for nursing neonates than solitary females, although there was not a significant difference in energy for nursing whether they had single or twin cubs. Supporting this result of maternal BW loss, the BW gain of single cubs was significantly different from that of twin cubs. This result shows that the energy given to a single cub during denning is greater than that given to one of twins, and the total energy loss of the mother is equalized. The milk produced by mother bears contains high fat and low sugar. This characteristic indicates that mother bears used the fat accumulated prior to denning for energy in raising cubs, and reduced their glucose consumption. Considering the results, these reproductive phenomena represent a remarkable efficiency in the use of energy for reproduction during the fasting period of Japanese black bears. Success of reproduction through such reproductive mechanism largely owe to the accumulation of energy by mothers before denning. The present study indicates that it is also important for us to preserve food production capacity of their habitat for conservation of wild population.

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