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Seasonal changes in the expression of energy metabolism-related genes in white adipose tissue and skeletal muscle in female Japanese black bears

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ABSTRACT

Bears undergo annual cycles in body mass: rapid fattening in autumn (i.e., hyperphagia), and mass loss in winter (i.e., hibernation). To investigate how Japanese black bears (*Ursus thibetanus japonicus*) adapt to such extreme physiological conditions, we analyzed changes in the mRNA expression of energy metabolism-related genes in white adipose tissues and skeletal muscle throughout three physiological stages: normal activity (June), hyperphagia (November), and hibernation (March). During hyperphagia, quantitative real-time polymerase chain reaction analysis revealed the upregulation of de novo lipogenesis-related genes (e.g., fatty acid synthase and diacylglycerol O-acyltransferase 2) in white adipose tissue, although the bears had been maintained with a constant amount of food. In contrast, during the hibernation period, we observed a downregulation of genes involved in glycolysis (e.g., glucose transporter 4) and lipogenesis (e.g., acetyl-CoA carboxylase 1) and an upregulation of genes in fatty acid catabolism (e.g., carnitine palmitoyltransferase 1A) in both tissue types. In white adipose tissues, we observed upregulation of genes involved in glyceroneogenesis, including pyruvate carboxylase and phosphoenolpyruvate carboxykinase 1, suggesting that white adipose tissue plays a role in the recycling of circulating free fatty acids via re-esterification. In addition, the downregulation of genes involved in amino acid catabolism (e.g., alanine aminotransferase) and the TCA cycle (e.g., pyruvate carboxylase) indicated a role of skeletal muscle in muscle protein sparing and pyruvate recycling via the Cori cycle. These examples of coordinated transcriptional regulation would contribute to rapid mass gain during the pre-hibernation period and to energy preservation and efficient energy production during the hibernation period.

1. Introduction

Mammalian hibernators undergo annual cycles in body mass: fattening in summer and/or autumn and mass loss in winter (Dark, 2005). Among hibernators, bears, including the Japanese black bear (*Ursus thibetanus japonicus*), have a unique hibernation physiology, surviving for up to 6 months without eating, drinking, urinating, or defecating (Hellgren, 1995), while exhibiting only a slight decrease in body temperature (Harlow et al., 2004; Shimozuru et al., 2013). In addition, in contrast to other hibernators, female bears give birth to cubs and nurse them during hibernation (Nelson, 1973). The energy required for fetal development, parturition, and lactation comes almost entirely from the fat reserves accumulated before hibernation (Nelson et al., 1983). Therefore, fat accumulation before hibernation and the efficient utilization of fat reserves during hibernation are crucial to survival and reproduction during the fasting period in female bears.

Annual physiological cycles in American black bears (*Ursus americanus*), Japanese black bears, and brown bears (*Ursus arctos*) can be divided into four stages: normal activity (from May to September), hyperphagia (from October to November), hibernation (from the end of November to April), and walking hibernation (the 2–3 weeks after emergence from hibernation) (Nelson et al., 1983). During the autumnal hyperphagia phase, a rapid increase in body mass is observed, especially in November, in both wild and captive bears, which parallels an increase in stored body fat (Nakamura et al., 2008; Yamanaka et al., 2011). The rapid mass gain during hyperphagia is mainly attributed to an increase in the availability of a nutrient-rich diet within the habitat, including nuts and acorns (Hashimoto, 2002), and the subsequent increase in daily food intake (Nelson et al., 1983). On the other hand, physiological changes have also been shown to contribute to fat accumulation during hyperphagia. Some studies have suggested that, during hyperphagia, bears enhance the efficiency of the hepatic conversion of dietary carbohydrate to triglycerides (TGs) by increasing the efficiency of carbohydrate and fat digestion (Brody and Pelton, 1988), by accelerating peripheral glucose uptake (Kamine et al., 2012), and by upregulating hepatic mRNA expression of enzymes involved in lipogenesis (Shimozuru et al., 2012b). However, it remains unknown whether this

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increased lipogenic activity during hyperphagia also occurs in other peripheral tissues, including white adipose tissue (WAT).

In contrast, during hibernation, bears lose approximately 25% of their body weight (Nelson, 1973). However, lean body mass remains constant during this period (Lundberg et al., 1976), and severe muscle atrophy and bone loss do not occur in hibernating bears (Harlow et al., 2001; McGee-Lawrence et al., 2009), although they are nearly immobile throughout hibernation. The respiratory quotient (RQ: the ratio of carbon dioxide liberated to oxygen consumed) decreases from 0.8 to near 0.7 (an RQ of 0.71 represents pure fat combustion) (Ahlquist et al., 1984; Fedorov et al., 2009; Nelson et al., 1973), suggesting that energy is produced mainly by body fat catabolism, and not by protein degeneration in skeletal muscle, during hibernation. During a state of negative energy balance, such as during hibernation and starvation, TGs stored in WAT are converted into glycerol and free fatty acids (FFAs), which are used for gluconeogenesis and amino acid formation in the liver, and as an energy source by peripheral tissues (i.e., β-oxidation), respectively. FFAs are also converted into ketone bodies in the liver for use as an energy source in other tissues, such as the brain, heart, and skeletal muscle. Previously, we found that the expression of hepatic genes involved in energy metabolism are regulated in coordination during hibernation in Japanese black bears: i.e., the downregulation of glycolysis-, amino acid catabolism- and de novo lipogenesis-related genes, and the upregulation of gluconeogenesis-, β-oxidation-, and ketogenesis-related genes (Shimozuru et al., 2012a). These modulations of hepatic gene expression would play a central role in the efficient production and utilization of energy by hibernating bears; however, other peripheral tissues could conceivably contribute to this process. For example, the maintenance of blood glucose levels during hibernation (Ahlquist et al., 1984) can be achieved not only by upregulation of gluconeogenesis in the liver, but also by downregulation of glycolysis and/or upregulation of the utilization of lipid-related metabolites (i.e., FFAs and ketone bodies) in other peripheral tissues, including the brain, heart, skeletal muscle, and adipose tissue. However, only a few studies have addressed this issue in bear species (Fedorov et al., 2009, 2011).

In the present study, we focused on seasonal changes in the energy metabolism of WAT, the largest energy-storing organ, and of skeletal muscle, the largest energy-consuming organ, in Japanese black bears. We hypothesized that, similar to what was suggested in previous studies of the liver (Fedorov et al., 2009, 2011; Shimozuru et al., 2012a, 2012b), the seasonal modulation of energy metabolism in WAT and skeletal muscle contributes to efficient fat accumulation during hyperphagia and to energy preservation and the efficient utilization of lipids during hibernation. To test this hypothesis in captive female Japanese black bears, the mRNA expression of energy metabolism-related genes in WAT and skeletal muscle were compared among three of four of the abovementioned physiological stages: normal activity (June), hyperphagia (November), and hibernation (March).

2. Materials and methods

2.1. Animals

A total of 20 adult female Japanese black bears, between the ages of 5 and 20, were studied. None of them were pregnant and they were kept at the Ani Mataginosato Bear Park located in Akita Prefecture, northeastern Japan (40°N, 140.1°E), from 2010 to 2013. During the active period, from late-April to late-November, the bears were provided dried corn (360 kcal/100 g, approximately 1.5 kg/head) once a day at 16:00 h, and water was provided ad libitum. They were also provided with ration for bears and boars (KS, Oriental Yeast Co., Ltd., Tokyo, Japan) and some fruit and vegetables as supplements intermittently. Two weeks prior to the onset of feeding deprivation in late November or early December, the ration was reduced to one-third. At the end of November or the beginning of December, the bears were moved to indoor rooms for denning, where they were kept in isolation without feeding until the middle of the following April. The animals were provided with straw for nesting and with water ad libitum. All procedures and animal care were conducted in accordance with the Guidelines of the Animal Care and Use of Hokkaido University and were approved by the Animal Care and Use Committee of Hokkaido University (Permit Number: JU9124).

2.2. Sample collection

Collection of blood and tissues was conducted in mid-June during the normal activity phase, in early November during the hyperphagia phase, and in early March during the hibernation phase. In June and November, each animal was isolated in an indoor room and provided dried corn (1.5 kg/head) at 16:00 h. Water was provided ad libitum during the isolation. After eating, the bear was fasted overnight (about 15–16 h) until the administration of anesthesia in the morning of the following day. In March, the bears were maintained without feeding and were sampled in the morning. Bears were immobilized using blow darts with an intramuscular administration of 3.0 mg/kg of a zolazepam HCL and tiletamine HCL cocktail (Zoletil®, Virbac; Carros, France) and 40 μg/kg of medetomidine HCL (Domitor®; Zenoaq, Japan) based on estimated body weight. After immobilization, bears were weighed and blood and tissues were collected as follows.

Blood samples were collected from the jugular vein into vacuum tubes. Heparin was used as the anticoagulant for the determination of TGs, total cholesterol, and total ketone bodies (total concentration of β-hydroxybutyric acid and acetoacetic acid). A mixture of sodium fluoride, heparin sodium, and ethylenediaminetetraacetic acid disodium salt was used as the anticoagulant for glucose determination. Blood was also collected into vacuum tubes without an anticoagulant for the isolation of serum to assess FFAs. The collected blood samples were centrifuged at 1880 $\times g$ for 10 min, and the separated serum and plasma samples were rapidly frozen on dry ice. The blood samples were packed with dry ice, transported to the laboratory, and stored at −80 °C until assayed.

For WAT and skeletal muscle collection, a section of hair on the lumbar area and inner aspect of the thigh, respectively, was shaved, and the skin was washed thoroughly with an antimicrobial cleaner (Isodine® Wash; Meiji Seika Kaisha, Tokyo, Japan). Subcutaneous WAT and a portion of the sartorius muscle (*musculus sartorius*) was then collected, and samples (approximately 100 mg/head) were immediately placed in RNAlater (Ambion; Austin, TX, USA), an RNA preservation solution, and were stored at −20 °C until assayed. When a bear was studied in successive seasons, samples were taken from the opposite side of the body. After all experiments were completed, meloxicam (Metacam® Boehringer; Ingelheim, Germany) was administered subcutaneously at 0.2 mg/kg for analgesia, and atipamezole HCL (Antisedan®; Zenoaq, Japan: 200 μg/kg) was administered intramuscularly as the medetomidine HCL antagonist to aid recovery.

In this study, only some bears could be sampled repeatedly; thus, the number of samples varied among the assays. Table 1 shows the sample types and collection month for each bear. For WAT, 11 females were studied, and 8, 8, and 7 samples were obtained in June, November, and March, respectively. For skeletal muscle, 17 females were studied, and 8, 6, and 6 samples were obtained in June, November, and March, respectively. Body weight measurements and blood sampling were performed when each or both tissue samples were collected. For the body weight and blood biochemistry data analysis, a total of 20 females, including 12 bears in June, 11 bears in November, and 11 bears in March, were used.

2.3. Blood biochemistry

Plasma concentrations of glucose, TGs, and total cholesterol were measured using an automatic blood analyzer (DRI-CHEM 7000; Fujifilm Medical Co., Ltd., Tokyo, Japan). Serum FFAs and plasma ketone bodies were assayed using commercial kits (NEFA C test; Wako, Osaka, Japan; and Ketorex Kit; Sanwa Chemical Company, Nagoya, Japan, respectively) according to the manufacturer's protocols.