Blood chemistry reference intervals of captive Asiatic black bears (*Ursus thibetanus*)

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ABSTRACT: Data on blood chemistry values can make fundamental contributions to our understanding of physiological changes. However, there is a lack of information regarding blood chemistry in Asiatic black bears (*Ursus thibetanus*). Thus, the objects of this study were to determine reference ranges for 29 blood chemistry variables, and to evaluate differences between age groups and between seasons. Blood samples (*n* = 138) were collected from 44 (20 males, 24 females; age range, 1–15 years) clinically healthy, captive Asiatic black bears (*Ursus thibetanus*) in the Republic of Korea. Young and adult bears showed significantly higher levels of creatinine and total cholesterol, and lower levels of blood urea nitrogen, blood urea nitrogen/creatinine ratio, lactate dehydrogenase and creatine kinase MB during hibernation compared to during non-hibernation. Adults also showed significantly higher levels of triglyceride, but lower levels of inorganic phosphorus, aspartate transaminase, alkaline phosphatase, high density lipoprotein cholesterol and creatine phosphokinase during hibernation than during non-hibernation. During hibernation, the urea nitrogen/creatinine ratio and levels of alkaline phosphatase, lactate dehydrogenase, and creatine phosphokinase in young bears were significantly higher than in adults, whereas creatinine levels were lower than in adults. During non-hibernation, the urea nitrogen/creatinine ratio and levels of calcium, alkaline phosphatase, lactate dehydrogenase, creatine phosphokinase and creatine kinase MB in young bears were significantly higher, whereas creatinine, total protein, albumin, gamma-glutamyl transferase and haemoglobin levels were lower than in adults. The results of this study provide reference values that will aid in understanding the physiology of Asiatic black bears and in assessing the health of these animals in captive environments.

Keywords: Asiatic black bear; reference ranges; hibernation; blood chemistry; captivity; health assessment

List of abbreviations

ALB = albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AMY = amylase, AST = aspartate aminotransferase, BUN = blood urea nitrogen, CKMB = creatine kinase MB, CPK = creatine phosphokinase, CRE = creatinine, CRP = C-reactive protein, DBIL = direct bilirubin, GGT = gamma-glutamyl transferase, GLU = glucose, Hb = haemoglobin, HDLC = high density lipoprotein cholesterol, IP = inorganic phosphorus, LDH = lactate dehydrogenase, TBIL = total bilirubin, TCHO = total cholesterol, TG = triglyceride, TP = total protein, U/C = blood urea nitrogen to creatinine ratio, UA = uric acid

Blood chemistry values can play a fundamental role in facilitating an understanding of physiological changes and in assessing potential health problems at both individual and population levels (Geffre et al. 2009; Friedrichs et al. 2012). Previously, blood chemistry data have been report-
ed for American black bears (*Ursus americanus*; Matula et al. 1980; Nelson et al. 1984; Schroeder 1987; Hellgren et al. 1993), brown bears (*Ursus arctos*; Nelson et al. 1983; Brannon 1985; Hissa et al. 1994; Huber et al. 1997; Graesli et al. 2014; Graesli et al. 2015) and polar bears (*Ursus maritimus*; Nelson et al. 1983; Derocher et al. 1990; Ramsay et al. 1991; Tryland et al. 2002). These studies described blood chemistry or haematology values in these species and discussed several physiological factors that could affect them. Although Asiatic black bears (*Ursus thibetanus*) are endangered and extensive efforts have been invested in restoration of the species in the Republic of Korea, reference ranges for blood chemistry have not been established. Some blood chemistry variables have been reported for Japanese black bears (*Ursus thibetanus japonicas*; Kamine et al. 2012a; Kamine et al. 2012b; Shimozuru et al. 2012), but those studies were mainly focused on metabolism, pregnancy or anaesthesia, and the number of parameters was limited. In this report, we describe the differences in blood chemistry values of captive Asiatic black bears by season and age in the Republic of Korea. To the best of our knowledge, this is the first study to establish reference ranges for blood chemistry in Asiatic black bears.

**MATERIAL AND METHODS**

**Animals.** The study protocol was approved by the Animal Care and Research Committee of the Species Restoration Technology Institute, Korea National Park Service (SRTI number 16-013). The Asiatic black bears (44 total; 20 males, 24 females; age range, 1–15 years) used in this study were kept in the Species Restoration Technology Institute of Korea National Park Service, Republic of Korea (35°14′20″N, 127°29′33″E). The study ran from 2005 to 2015.

The bears were provided with commercial dried pellets (Omnivore Diet Dry, ZuPreem, Shawnee Mission, USA), various seasonal fruits, vegetables and chestnuts once a day with water provided *ad libitum* from late March to early December. Limited food was given to bears in mid-December, with no food in the last week, to induce hibernation in an artificial winter den. Each bear hibernated separately in its own winter den without food until the middle of the following March, although drinking water remained accessible. Feeding was resumed after the bears emerged from their dens.

**Sample collection and blood chemistry analysis.** Bears were immobilised intramuscularly with 2 mg/kg tiletamine/zolazepam (Zoletil 50®, Virbac, France) and 0.04 mg/kg medetomidine (Dormitor®, Pfizer, Finland) by remote delivery (PI CO₂, DanInject ApS, Denmark). After immobilisation, blood samples were collected from the femoral or jugular vein into a vacuum tube containing lithium heparin (*G-TUBE™*, Green Cross MS, Republic of Korea), with a 10-ml syringe with an 18- or 20-gauge needle. Samples were stored at 4 °C and analysed within 24 hours at the Wildlife Medical Centre of the Korea National Park Service, Republic of Korea. Twenty-nine blood chemistry values were analysed using a blood chemistry analyser (DRI-CHEM 3500i, Fuji, Japan). Blood chemistry tests were carried out for glucose (GLU), blood urea nitrogen (BUN), creatinine (CRE), blood urea nitrogen to creatinine (*U/C*) ratio, total cholesterol (TCHO), NH₃, total bilirubin (TBIL), Ca, inorganic phosphorus (IP), total protein (TP), albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), amylase (AMY), Na, K, Cl, C-reactive protein (CRP), direct bilirubin (DBIL), haemoglobin (Hb), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), alkaline phosphatase (ALP), creatine phosphokinase (CPK), creatine kinase MB (CKMB), Mg and uric acid (UA).

**Statistical analysis.** Samples were categorised by season (hibernation, late December to mid-March; non-hibernation, late March to mid-December) and by bear age (young, less than four years; adult, greater than or equal to four years). Blood samples were collected 2–4 times, on average, per individual during the study, but only one sample was used for data analysis if the samples were collected in the same year. In total, 138 samples were analysed in this study. All statistical analyses were performed with IBM SPSS Statistics 18® (IBM Corp., North Castle, USA). Results were tested for conformance to a normal distribution using the Shapiro-Wilk test, and outliers (greater than three standard deviations from the mean) were removed from the analyses. A Student’s *t*-test (two groups of greater than or equal to 30 cases) and a Mann-Whitney *U*-test (two groups of less than 30 cases) were performed to compare differences between age groups.
and between seasons. A level of $P < 0.05$ was considered statistically significant.

RESULTS

There were significant differences in the parameters between hibernation and non-hibernation within each age group. Seasonal differences were found in six out of 29 blood chemistry variables among young bears, and in 11 out of 29 variables among adults. The CRE and TCHO values in young bears during hibernation were significantly higher ($P < 0.001$ and $P = 0.002$, respectively), whereas those for BUN, U/C ratio, LDH, and CKMB were lower ($P = 0.002$, $P < 0.001$, $P = 0.008$, and $P = 0.004$, respectively) than during non-hibernation. The values of CRE, TCHO, and TG in adults during hibernation were significantly higher ($P < 0.001$, $P = 0.019$, and $P < 0.001$, respectively), whereas those for BUN, U/C ratio, IP, AST, ALT, LDH, HDLC and CPK were lower ($P < 0.001$, $P < 0.001$, $P = 0.002$, $P < 0.001$, $P < 0.001$, $P < 0.001$, and $P = 0.001$, respectively) than during non-hibernation. All results for blood chemistry variables are presented in Table 1.

Age-related differences were found in five out of the 29 blood chemistry variables during hibernation and in 11 out of 29 variables during non-hibernation. During hibernation, the values for the U/C ratio, ALT, LDH and CPK in young bears during hibernation were significantly higher ($P = 0.024$, $P = 0.002$, $P = 0.023$, and $P = 0.031$, respectively), whereas those for CRE were lower ($P = 0.001$), than in adults. Further, the values of the U/C ratio, Ca, ALP, LDH, CPK and CKMB in young bears during non-hibernation were significantly higher ($P < 0.001$, $P = 0.005$, $P < 0.001$, $P < 0.001$, $P = 0.012$, and $P < 0.001$, respectively), whereas those for CRE, TP, ALB, GGT and Hb were lower ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.003$, and $P = 0.002$, respectively), than in adults.

There were no significant differences in the values of GLU, NH$_3$, TBIL, DBIL, AMY, electrolytes, CRP and UA between seasons, or between ages.

DISCUSSION

Seasonal changes in blood chemistry values in bears are generally a response to changes in physiological conditions caused by nutrition, metabolic rate and stress. Bears undergo notable metabolic changes during hibernation, although many of the mechanisms regulating hibernation remain to be elucidated. Hibernating bears mainly metabolise stored fat reserves because they ingest no food or water for months, and their urea production decreases partly owing to the shift from protein to fat metabolism during hibernation (Graesli et al. 2015).

CRE values were significantly higher during hibernation than during non-hibernation in both young and adult bears. CRE is good indicator of renal function, with high values indicating impaired function (Stockham and Scott 2008). During hibernation, the glomerular filtration rate (GFR) in bears decreases by about 70% (Brown et al. 1971). Besides, CRE is not metabolised and is cleared only by the kidney. Taken together, this seems to result in increased CRE values during hibernation. These results are similar to those from previous studies in hibernating brown and American black bears (Schroeder 1987; Hellgren et al. 1993; Hissa et al. 1994). The values of CRE were significantly higher in adults compared to young bears, regardless of season. This may be due to the change in muscle size with age. Brannon (1985) attributed this higher CRE in adult bears to larger muscle mass.

BUN values were significantly lower during hibernation than during non-hibernation in both young and adult bears. This difference between seasons was probably caused by seasonal fluctuations in the availability of protein-containing foods. Since urea is a product of protein metabolism, differences in protein content in the diet might be reflected in the blood values of urea (Graesli et al. 2014). Thus, starvation during hibernation seems to be a primary reason for the decrease in BUN levels. Another possible reason is the synthesis and the resorption of urea. Nelson (1980) reported that the urea formed during the catabolism of proteins is hydrolysed in the intestinal tract, and the nitrogen formed re-enters protein synthetic pathways. This process is faster than the synthesis of urea (Nelson et al. 1975; Nelson 1980).

The value of the U/C ratio significantly decreased during hibernation in all bears in this study. This is probably due to reduced protein breakdown, decreased renal filtration and elevated urea hydrolysis (Barboza et al. 1997; Hissa et al. 1998). In addition, higher CRE values, which may be due to decreased
renal filtration and muscle proteolysis, can explain the lower blood U/C ratio during hibernation. The higher U/C ratio in young bears also seems to be related to the amounts of CRE in muscle mass based on age.

The elevated TCHO and TG during hibernation in all bears in our study reflect lipolysis from adipose tissue; this catabolism of fat supplies the energy for basal metabolism. Our results were similar to those reported in studies of other bear species (Lohuis et al., 2015).
hibernation. This seems to be due to the differences in primary foods and dietary protein levels between young and adult bears, and it shows that the adults were in better physiological condition. TP and ALB values are indicators of dietary protein levels, and Hellgren et al. (1993) previously documented a correlation between primary food source and TP values. Furthermore, Matula et al. (1980), in the American black bear, also reported differences in Hb values by age which were correlated with diet and growth. However, because food availability during hibernation is limited for both young and adult bears, there may not be any differences in TP, ALB and Hb values between ages.

In adult bears, the IP values were significantly lower during hibernation, and there were no changes in blood Ca values during the hibernation season compared to during non-hibernation. During hibernation, the lower value of blood IP reflects a state of starvation and reduced caloric intake with redistribution from the extracellular to intracellular space when cellular phosphate demand is insufficient. This is also a bone-preserving mechanism (McGee-Lawrence et al. 2009; Vestergaard et al. 2011). These results are different from those for humans and have been thought to reflect the special physiology of bears (Stenvinkel et al. 2013a).

The ALP and Ca values in young bears during non-hibernation were higher than those in adults. ALP exists in different isoenzymatic forms and is responsible for the hydrolysis of monophosphate esters in different tissues. The isoenzyme from bone marrow is produced by osteoblasts, and blood levels of this form in young and rapidly growing animals might be three times higher than those of adults (Duncan et al. 1994). High values of blood Ca reflect the rapid growth of bone in young bears during non-hibernation. The results of this study correspond with those of previous studies on American black bears from Pennsylvania (Storm et al. 1988) and wild polar bears from Canada (Lee et al. 1977).

In this study, the Mg values in young bears during non-hibernation were significantly higher than those in adults. This is probably because only juvenile mammals can mobilise Mg from the skeleton when food intake is inadequate (Rosol and Capen 1997).

Enzyme-related variables (ALT, AST and LDH) in adults tended to decrease during hibernation. This may be due to the shift from carbohydrate and protein metabolism to fat metabolism. These physiological changes result in decreased urea production and reduced demand for the enzymes important for protein breakdown (Graesli et al. 2015).

The LDH values of young bears were significantly higher than those of adults in our study. High levels of LDH in blood are usually associated with general cell damage or necrosis (Bossart et al. 2001). The higher levels of LDH found in young individuals may be influenced by the fact that young bears were immobilised subsequent to their mothers, and thus experienced a longer period of stress and muscle activity before immobilisation (Tryland et al. 2002).

The CPK and CKMB values in adults were lower than those of young bears in this study. These variables are generally correlated with cardiac or skeletal muscle injuries, and higher CPK and CKMB levels in young individuals may therefore be a result of the increased stress and muscle stiffness before immobilisation compared to adult bears.

The GGT value tended to increase with age, although the difference between that of young and that of adult bears was significant only during non-hibernation. In general, an increased GGT level indicates that the liver is damaged but does not specifically point to a condition that may be causing the injury. However, we could not find any clinical evidence of liver disease, and the GGT values in our study are similar to those of other bear species (Storm et al. 1988; Graesli et al. 2015).

There were no differences the level of GLU by season and age in this study, although it is generally influenced by stress or metabolic rate. These results are similar to those reported in previous bear studies (Wright et al. 1999; Graesli et al. 2014). Fat is the preferred source of energy during hibernation, while carbohydrates and proteins are spared (Chauhan et al. 2002). However, GLU also plays a central role in carbohydrate metabolism, and when carbohydrate stores decrease below normal...
during hibernation, glucose can be formed from amino acids and from glycerol via gluconeogenesis (Wright et al. 1999). These phenomena may ensure the preservation of a certain level of GLU regardless of season.

The mechanism of NH₃ generation during hibernation is not completely clear, but has been postulated to result from the passage of urea into the intestine, where it is hydrolysed by urease-expressing gut bacteria to release ammonia (Nelson et al. 1975; Barboza et al. 1997). Although some of the ammonia may be used by the gut bacteria for their own growth (Wright 1995), the remainder is absorbed where it can react with glycerol released during the lipolysis of fat to make amino acids (Stenvinkel et al. 2013b). The fact that ammonia levels do not increase during dormancy underlines the efficient recycling of ammonia into various amino acids, especially glutamine (Wright et al. 1999). Thus, NH₃ values during the hibernation period seem to not differ greatly from those in non-hibernation.

In this study, UA values tended to be lower during hibernation as compared with those in non-hibernation, although there was no statistically significant difference between seasons. Stenvinkel et al. (2013a) reported that hibernating brown bears had significantly lower UA and this decrease indicated a reduced UA catabolism during hibernation, while Wright et al. (1999) described UA values in American black bears to be unchanged by season. Thus, species differences probably play a role and further study is needed.

There were no significant differences in the values of electrolytes (Na, K, Cl), in common with previous studies in American black bears (Hellgren et al. 1990; Lohuis et al. 2005), between seasons in the present study. This was probably due to essential kidney function which maintained the volume and composition of bodily fluids and the special physiology of bears, i.e., a reduction in glomerular filtration rate and reabsorption of filtrate by the kidney and bladder (Jani et al. 2013).

AMY is a starch hydratase and its levels tended to be lower in hibernating bears, although there was no statistically significant difference in this study. The decrease in protein breakdown and the inhibition of gluconeogenesis during hibernation may explain the lower enzyme levels during winter.

The TBIL values described by Storm et al. (1988) and Matula et al. (1980) for American black bears (5.1 and 3.42 µmol/l, respectively) were below our median values (8.4–8.6 µmol/l), but the values in European brown bears (Huber et al. 1997) were higher (12.4 µmol/l) than our results. Matula et al. (1980) reported TBIL levels in cubs and yearling bears to be significantly higher than in adults and speculated that younger animals may be more susceptible to erythrocyte damage or hepatic alterations caused by immobilisation and handling techniques. Besides, Lohuis et al. (2005) reported higher TBIL values during hibernation in American black bears compared to values in summer. However, in our study, there were no significant differences by age or by season.

CRP is a non-specific inflammatory marker and in humans reduced renal function is commonly associated with low-grade persistent inflammation (Carrero and Stenvinkel 2010). A previous study in bears has shown that glomerular filtration rate (GFR) is reduced to about only one fourth of its normal value during hibernation (Brown et al. 1971). Thus, considering this relationship between reduced renal function and inflammation, the value of CRP would be expected to increase in the hibernation period. However, in the present study CRP values did not show any seasonal variations and these results are similar with previous data from brown bears (Mominoki et al. 2005; Stenvinkel et al. 2013a). This indicates that bears do not develop systemic inflammation during hibernation in spite of reduced renal function and prolonged inactivity.

Our results establish clinical blood chemistry reference ranges for evaluation of the health of both hibernating and non-hibernating captive Asiatic black bears. The reference values we obtained will be helpful in understanding the physiology of Asiatic black bears. However, further studies on haematological and blood chemistry responses to disease are still needed as a tool in the assessment of pathological conditions.

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