DOI: 10.7589/2017-08-201
Journal of Wildlife Diseases, 54(3), 2018, pp. 575–580

Blood Chemistry Reference Values for Free-Ranging Asiatic Black Bears (*Ursus thibetanus*) by Season, Age, and Sex

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ABSTRACT: Physiological characteristics, such as blood chemistry values, are valuable for evaluating the health of the animals. To our knowledge, these values have never been reported for the free-ranging Asiatic black bear (*Ursus thibetanus*; ABB). Thus, 28 blood chemistry values from 50 free-ranging ABBs captured in Jirisan National Park, Republic of Korea, from 2005 to 2016 were evaluated. The aim of this study was to establish blood chemistry reference values for the free-ranging ABBs during both the hibernating and nonhibernating seasons. During hibernation, mean values of creatinine (CRE), total cholesterol, total protein (TP), albumin (ALB), triglycerides, and magnesium were significantly higher than those during nonhibernation; however, mean values of blood urea nitrogen, urea nitrogen to creatinine (U/C) ratio, inorganic phosphorous (IP), aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, lactate dehydrogenase (LDH), high-density lipoprotein cholesterol, and alkaline phosphatase (ALP) were significantly lower. Age differences (young vs. adult) were found in IP, LDH, TP, and ALB values during hibernation and in the U/C ratio, calcium, IP, ALP, creatine kinase myocardial band, CRE, total bilirubin, and uric acid values during nonhibernation. However, there were no sex differences (male vs. female).

Key words: Asiatic black bear, blood chemistry, health assessment, hibernation, reference values, *Ursus thibetanus*.

The Asiatic black bear (*Ursus thibetanus*; ABB) contains seven subspecies and is distributed through much of southern Asia, northeastern China, far eastern Russia, and Japan (Servheen 1989). However, populations are globally decreasing, and the bear is almost extinct in the Republic of Korea. A restoration project for ABBs has been ongoing in Korea since 2001 to recover the bear numbers, and an understanding of its physiological characteristics is necessary for the project's success. Blood chemistry values are fundamental for evaluating the health status of free-ranging animals (Barnes et al. 2008) and are responsive to acute or chronic diseases. However, reference values for blood chemistry in free-ranging ABBs have not been established. The objectives of this study were to establish reference values for blood chemistry parameters of free-ranging ABBs and to confirm whether there are differences in these parameters due to season, age, or sex.

The study area, Jirisan National Park, is located in the southern part of the Korean peninsula (35°12′40″–35°26′40″N, 127°27′20″–127°49′40″E; 472 km^2) and is the largest protected area in the Republic of Korea. The study included 50 free-ranging ABBs (26 males and 24 females, 1 to 11 yr old), caught and anesthetized for health examination, attachment of radio transmitters, and translocation between 2005 and 2016. Bears were captured using a baited culvert trap during the active season, and winter den capturing was employed during the hibernation period. Prior to blood collection, bears were anesthetized with 2 mg/kg tiletamine-zolazepam (Zoletil 50®, Virbac, Carros, France) and 0.04 mg/kg medetomidine (Dormitor®, Pfizer, Espoo, Finland) intramuscularly using a CO_2-powered immobilizing gun (CO_2 PI, Dan-Inject Aps, Børkop, Denmark). Venous blood samples were collected from the femoral or jugular vein into a 10-mL syringe with a sterile, 20-ga needle. The samples were immediately placed in vacutainers containing anticoagulant (K3EDTA VACUETTE®, Greiner Bio-One, Monroe, North Carolina, USA) and lithium heparin (G-TUBE™,

TABLE 1. Reference values for 28 blood chemistry parameters in free-ranging Asiatic black bears (*Ursus thibetanus*) in Jirisan National Park, Republic of Korea, 2005–16.

	Hibernation (late December to mid-March)				Nonhibernation (late March to mid-December)			
Parameter (unit)[a]	*n*	Mean (median)±SD	95% CI	Range	*n*	Mean (median)±SD	95% CI	Range
Age (yr)	38	4.6 (4.5)±2.2	3.9–5.4	2–9	119	3.6 (3)±2.6	3.1–4.1	1–11
Weight (kg)	38	75.5 (65.5)±37.6	63.6–87.9	16–168	119	58.2 (50)±40.2	50.8–65.7	10–150
GLU (mmol/L)	37	4.9 (5.2)±1.4	4.3–5.5	2.2–7.7	119	6 (5.9)±2.4	5.2–6.9	1.6–12.7
BUN (mmol/L)[b]	38	0.8 (0.6)±0.9	0.4–1.3	0–3.5	116	2.1 (1.4)±2.2	1.4–2.8	0–10.6
CRE (μmol/L)[b,c]	38	229.3 (247.5)±79.7	189.7–267.9	26.5–406.6	119	100.9 (106.1)±44.6	86–114.9	26.5–203.3
U/C[b,c]	38	3.7 (2.4)±4.2	1.8–5.9	0.1–14	115	25.1 (14.9)±26	16.2–33.9	0.3–85.5
TCHO (mmol/L)[b]	37	9.4 (9.5)±1.8	8.5–10.2	5.7–11.7	117	7.8 (7.9)±1.5	7.3–8.3	4.7–10.7
NH_3 (μmol/L)	27	41.5 (36.1)±25.5	31.5–54.3	15.8–101	84	43.6 (38.7)±16.6	38.6–49.6	19.4–91
TBIL (μmol/L)	38	6.3 (6)±3.7	4.7–8	1.7–13.7	115	5.6 (5.1)±3.5	4.5–6.8	1.7–13.7
Ca (mmol/L)[c]	35	2.1 (2.1)±0.1	2.1–2.2	1.9–2.4	119	2.1 (2.1)±0.2	2–2.2	1.7–2.6
IP (mmol/L)[b,c,d]	38	1.3 (1.3)±0.2	1.2–1.4	0.8–1.7	117	1.9 (1.9)±0.6	1.7–2.1	0.9–3.1
TP (g/L)[b,d]	37	73.7 (74.5)±4	71.9–75.5	66–79	117	68.2 (68.5)±4.6	66.7–69.7	59–77
ALB (g/L)[b,d]	38	42.3 (41)±5.2	39.9–44.6	35–50	118	37.1 (38)±5.3	35.3–38.9	26–48
AST (μkat/L)[b]	32	0.7 (0.7)±0.1	0.7–0.8	0.7–1	107	0.9 (0.9)±0.3	0.8–1.1	0.5–1.7
ALT (μkat/L)[b]	38	0.2 (0.2)±0.1	0.2–0.2	0.1–0.5	118	0.4 (0.4)±0.1	0.4–0.5	0.2–0.7
GGT (μkat/L)[b]	30	0.4 (0.4)±0.1	0.4–0.5	0.3–0.6	94	0.6 (0.5)±0.2	0.5–0.6	0.2–1.2
LDH (μkat/L)[b,d]	30	6.3 (6.1)±1	5.8–6.8	5–9.1	100	10.9 (10.4)±2.6	10–11.8	6.2–15
AMY (μkat/L)	38	0.4 (0.4)±0.2	0.3–0.5	0–0.7	115	0.6 (0.4)±0.5	0.4–0.8	0.1–1.9
Na (mmol/L)	29	135 (137)±8.1	131–138.4	119–146	97	137.5 (140)±5.8	135.6–139.3	119–145
K (mmol/L)	29	4.1 (4.1)±0.6	3.8–4.4	3.2–5.1	94	4 (4)±0.5	3.8–4.1	3–5
Cl (mmol/L)	28	105.5 (106)±9.3	100.8–109.5	84–119	98	106.2 (107)±7.3	103.9–108.5	92–123
CRP (nmol/L)	27	10 (3.8)±9	6–14.3	2.9–27.6	91	9.2 (4.8)±6.9	6.8–11.6	2.9–20
DBIL (μmol/L)	30	2.8 (1.7)±4.4	1.7–5	1.7–20.5	92	2.9 (1.7)±3.6	1.9–4.4	1.7–17.1
HDL-C (mmol/L)[b]	30	1.9 (2.1)±0.98	1.4–2.3	0.3–2.8	96	2.8 (2.8)±0.2	2.7–2.8	1.8–2.8
TG (mmol/L)[b]	29	3.8 (3.8)±1.3	3.3–4.4	1.6–5.7	101	2 (1.9)±0.8	1.7–2.3	0.8–4.4

TABLE 1. Continued.

Parameter (unit)[a]	Hibernation (late December to mid-March)				Nonhibernation (late March to mid-December)			
	n	Mean (median)±SD	95% CI	Range	n	Mean (median)±SD	95% CI	Range
ALP (µkat/L)[b,c]	37	2.1 (1.8)±1.1	1.6–2.6	0.7–4.5	114	3.2 (3)±1.7	2.6–3.8	0.3–7
CPK (µkat/L)	30	1.6 (1.3)±0.9	1.3–2.1	0.7–4.8	97	2.1 (1.8)±1.1	1.8–2.5	0.8–5.3
CKMB (µkat/L)[c]	30	2.8 (2.3)±1.4	2.3–3.5	1.1–5	98	2.8 (2.7)±1.4	2.4–3.3	0.8–5
Mg (mmol/L)[b]	30	0.8 (0.8)±0.1	0.8–0.9	0.7–1	99	0.7 (0.7)±0.1	0.7–0.8	0.6–1
UA (µmol/L)[c]	30	51.5 (50.6)±13.5	46–58.4	23.8–89.2	98	52.8 (50.6)±17.8	47.1–58.6	23.8–95.2

[a] CI = confidence interval; GLU = glucose; BUN = blood urea nitrogen; CRE = creatinine; U/C = blood urea nitrogen to creatinine ratio; TCHO = total cholesterol; NH_3 = ammonia; TBIL = total bilirubin; Ca = calcium; IP = inorganic phosphorus; TP = total protein; ALB = albumin; AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGT = gamma-glutamyl transferase; LDH = lactate dehydrogenase; AMY = amylase; Na = sodium; K = potassium; Cl = chloride; CRP = C-reactive protein; DBIL = direct bilirubin; HDL-C = high density lipoprotein cholesterol; TG = triglyceride; ALP = alkaline phosphatase; CPK = creatine phosphokinase; CKMB = creatine kinase myoglobin; Mg = magnesium; UA = uric acid.

[b] Significant difference ($P<0.05$) between hibernation and nonhibernation.

[c] Significant difference ($P<0.05$) between young bears and adults during nonhibernation.

[d] Significant difference ($P<0.05$) between young bears and adults during hibernation.

Green Cross MS, Yongin, Republic of Korea). Other biological samples (including hair, feces, skin, and saliva) were also collected and were immediately air-shipped on ice to the Wildlife Medical Center of the Korea National Park Service (Gurye, Republic of Korea). Then serological screening for 12 infectious diseases including rabies, heart worm, canine distemper, and examination for parasites was conducted. Physical examination was also done, and only clinically healthy bears were included in this study.

Blood samples were collected two to four times per individual during the study, but only one sample per bear per year was included in data analysis. Thus, the interval of samples used in analysis for a bear was at least more than one year, and, when more than one sampling was done in a year, only the first sample collected was used because there was no significant difference in values between the samples. A total of 28 blood chemistry parameters were analyzed using a blood chemistry analyzer (DRI-CHEM 3500i, Fuji, Tokyo, Japan). Blood chemistry tests were conducted for glucose, blood urea nitrogen (BUN), creatinine (CRE), blood urea nitrogen to creatinine (U/C) ratio, total cholesterol (TCHO), ammonia, total bilirubin, calcium (Ca), inorganic phosphorus (IP), total protein (TP), albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), amylase, sodium, potassium, chloride, C-reactive protein, direct bilirubin, high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), alkaline phosphatase (ALP), creatine phosphokinase, creatine kinase myocardial band (CKMB), magnesium (Mg), and uric acid (UA). The samples were categorized by season (hibernation: late December to mid-March; nonhibernation: late March to mid-December), age (young: $<$4 yr; adult: $\geq$4 yr), and sex (male; female). A total of 157 blood samples were analyzed. Results were tested for conformance to a normal distribution with a Shapiro-Wilk test. A Student's t-test (two groups of $\geq$30 cases) and a Mann-Whitney U-test (two groups of $<$30 cases) were performed to compare differences between seasons, sexes, and ages. All statistical analyses were performed with IBM SPSS Statistics v. 18® (IBM Corp., North Castle, New York, USA). An alpha level of <0.05 was used for statistical comparisons.

Seasonal differences (hibernation vs. nonhibernation) were found in 15 of 28 blood chemistry parameters in all bears (Table 1). During hibernation, creatinine (t=7.48, P<0.001), TCHO (t=3.21, P=0.002), TP (t=4.26, P<0.001), ALB (t=3.39, P=0.001), TG (t=5.44, P<0.001), and Mg (t=2.02, P=0.048) were significantly higher than they were during nonhibernation. However, in hibernation, BUN (t=–2.88, P=0.006), U/C ratio (t=–4.69, P<0.001), IP (t=–5.52, P<0.001), AST (t=–3.43, P=0.001), ALT (t=–7.16, P<0.001), GGT (t=–3.28, P=0.002), LDH (t=–9.12, P<0.001), HDL-C (t=–4.17, P=0.001), and ALP (t=–2.45, P=0.018) were significantly lower than they were during nonhibernation. Age differences (young vs. adult) were found in four blood parameters during hibernation and seven blood parameters during nonhibernation. In young bears, IP (1.5±0.1 mmol/L vs. 1.2±0.2 mmol/L, P=0.037) and LDH (7.5±1.2 µkat/L vs. 5.9±0.7 µkat/L, P=0.037) were significantly higher, whereas TP (72.0±3.2 g/L vs. 74.9±4.3 g/L, P=0.037) and ALB (40.9±5.6 g/L vs. 45±3.5 g/L, P=0.037) were lower than they were in adults during hibernation. In addition, the U/C ratio (34.7±27.9 vs. 9.7±11.8, P=0.001), Ca (2.2±0.2 mmol/L vs. 2.0±0.1 mmol/L, P=0.027), IP (2.1±0.6 mmol/L vs. 1.7±0.5 mmol/L, P=0.038), ALP (4±1.6 µkat/L vs. 2±0.8 µkat/L, P<0.001), and CKMB (3.5±1.3 µkat/L vs. 1.7±0.6 µkat/L, P<0.001) in young bears were significantly higher than in adult bears, whereas CRE (78.7±33.4 µmol/L vs. 136.7±36.9 µmol/L, P<0.001) and UA (47.9±15.6 µmol/L vs. 60.9±18.8 µmol/L, P=0.037) were lower than they were in adults during nonhibernation. There were no differences between sexes in both seasons.

Higher mean CRE and Mg values during hibernation in this study were probably due to a reduced glomerular filtration rate. Brown et al. (1971) found that the glomerular filtration rate of hibernating bears is reduced by approximately 70%. Similar results have also been documented for brown bears (*Ursus arctos*) and American black bears (*Ursus americanus*) during hibernation (Schroeder 1987; Hellgren et al. 1993; Hissa et al. 1994). Increased TCHO and TG and decreased BUN during hibernation may be related to the conversion of energy sources. In the active season, the bears mainly use protein as an energy source, while during hibernation they use stored fat (Arinell et al. 2012; Græsli et al. 2015). This change results in a decrease of BUN and the U/C ratio. A reduction in the breakdown of protein would also cause a decrease in enzymes such as ALT, AST, GGT, and LDH during hibernation. Decreased IP during hibernation in this study may reflect a state of starvation and reduced caloric intake due to a redistribution of phosphate from extracellular to intracellular space when cellular phosphate demand is insufficient (Stenvinkel et al. 2013).

Physiological differences between young and adult bears may be influenced by growth and food sources. Lower TP and ALB in young bears during hibernation, in this study, may be attributed to less variation and a lesser amount of food in the diet compared to adults, and these results are like those of previous studies on American black bears and brown bears (Hellgren et al. 1993; Græsli et al. 2015). These age-related differences in diet also seem to affect UA, because UA can be generated from the catabolism of purines and fruits (Stenvinkel et al. 2013), and adult bears could consume more fruits, such as berries, than young bears. Thus, this probably results in higher UA values in adults, and Græsli et al. (2015) also reported higher UA values in adult brown bears. However, because the information about age-related food resources of ABBs in Korea is limited and the relationship between UA and age is unclear in bear species, further studies are required. Alternatively, ALP and IP were higher in young bears, which probably reflected elevated osteoblastic activity that is known to occur during rapid bone growth (Stockham and Scott 2008). Schroeder (1987) and Storm et al. (1988) reported similar results for American black bears. Higher concentrations of Ca in young bears in our study likely indicated rapid bone growth during nonhibernation, and this corresponds with the results of previous studies in American black bears (Storm et al. 1988). Higher CRE in adults may be due to greater muscle mass because CRE is primarily stored in muscles. The higher concentrations of LDH and CKMB in young bears are related to muscle damage or necrosis and probably reflected greater muscle damage compared to adults. Although the mechanisms underlying these relationships are unclear, many previous studies (Bossart et al. 2001; Tryland et al. 2002; Græsli et al. 2015) have presented similar results. Our study may have a limitation in that the bears were captured repeatedly, and negative memories regarding those events could potentially have induced stress such as anxiety or fear. However, because the level of stress is different in each animal, it is difficult to know how much stress affected physiologic body condition. Thus, in this kind of study, it would be probably helpful to confirm the change of glucocorticoid hormone, which is an indicator of stress.

Measures of serum chemistry parameters are important diagnostic tools for assessing animal health, but to be interpreted accurately, clinical pathology information collected during examinations must be compared to reference intervals from healthy animals, as many baseline biochemical parameters are influenced by factors such as age, diet, and environment (Beechler et al. 2009). Although the number of animals in our study has limitations, to the best of our knowledge, this is the first report of blood chemistry reference values for free-ranging ABBs during both hibernation and nonhibernation. The data presented here will enable managers and veterinarians to better care for ABBs and will serve as the foundation for physiologic studies in ABBs.

The authors thank the staff of the Species Restoration Technology Institute of the Korea National Park Service for their assistance. This study was conducted as a part of the Endangered Species Restoration Project of the Ministry of Environment, Republic of Korea, and complied with the current laws of the Republic of Korea.

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Submitted for publication 20 August 2017.
Accepted 21 January 2018.