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BLOOD CHEMISTRY OF BLACK BEARS FROM PENNSYLVANIA DURING WINTER DORMANCY

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ABSTRACT: Twenty-four serum chemistries were measured in blood samples collected from 20 adult female black bears (*Ursus americanus*) and their offspring, including 14 yearlings and 37 cubs, in northeastern Pennsylvania during winter 1984. Four other captive adult females were bled before, during, and after they were subjected to unseasonably warm temperatures during February. Levels of serum urea nitrogen (SUN) and creatinine were lower ($P < 0.05$), and iron was higher ($P < 0.05$) in male cubs compared to female cubs; serum chemistries were similar ($P \geq 0.05$) between sexes for yearlings. Total protein, albumin and creatinine levels increased with age of bears, whereas chloride, alkaline phosphatase, potassium, inorganic phosphorus and SUN/creatinine were higher ($P < 0.05$) in cubs than in yearlings and adults. The relatively high serum calcium in cubs was probably related to rapid bone development and dietary intake of calcium during winter dormancy in cubs. Low serum calcium in adults was attributed to lactation and a lack of dietary intake. Urea/creatinine ratios averaged 5.5 and 4.6 for yearling females and males, respectively, 6.3 for adult females, and 29.0 and 22.8 for female and male cubs, respectively. Levels of serum chemistries of black bears apparently are relatively stable during winter denning, when bears are without food or water and do not urinate or defecate for several months. This stability indicates that black bears are resistant to the extremes in extrinsic environmental conditions. Abnormal blood chemistry values may indicate metabolic stresses that are not being controlled by bears. Three of four captive bears subjected to warm temperatures in February showed a decrease in glucose and SUN and apparently were dehydrated during the temperature crisis when the animals became restless and attempted to escape. The reference values presented in this report should be useful to develop and evaluate health profiles of black bears in various ecological conditions.

Key words: Age and sex variation, black bear, blood chemistry, physiology, *Ursus americanus*, winter dormancy.

INTRODUCTION

Metabolic profiles derived from blood can be used to assess the health and physiological status of black bears (*Ursus americanus*) during periods of confinement as well as aid in diagnosis of disease, stress, and malnutrition of bears in zoos, research centers and free-ranging situations (Seal et al., 1981). A limited amount of data on blood has been reported for captive and free-ranging bears (King et al., 1960; Erickson and Youatt, 1961; Seal et al., 1967; Nelson et al., 1973; Eubanks et al., 1976). Matula (1976) presented reference blood values for black bears during predenning (July to December) and post-

denning (late April to July) in Pennsylvania. However, data on blood chemistries of black bears in Pennsylvania during winter dormancy (December to early April) have not been published. During denning, yearling and adult bears do not eat, drink, urinate or defecate. Also during this period, adult females support fetal development, experience parturition and nurse their offspring. This report presents (1) reference blood chemistry values for cub, yearling and adult female black bears during winter dormancy, (2) compares blood values of cub and yearling bears in winter to those reported for non-winter months in Pennsylvania, and (3) presents data on

physiological changes in stressed captive bears.

MATERIALS AND METHODS

From February to early April 1984, blood samples were obtained from 20 adult female black bears occupying winter dens in north-eastern Pennsylvania (41°15' to 41°45'N, 74°45' to 75°30'W). Fourteen females had nursing cubs, and six were with yearlings. Blood samples were collected also from 37 cubs (17 females and 20 males) and 14 yearlings (10 females and four males) associated with the 20 adult females.

Six other adult females were maintained with their respective cubs in separate cages on both levels (four on upper floor and two on ground floor) of an unheated, two-story barn in north-eastern Pennsylvania during January and February 1984. These six adults did not eat during captivity. Ambient temperatures increased markedly during the second week of February. The daytime high temperatures averaged 1 C during 5 to 11 February compared to 11 C during 12 to 18 February. As the ambient temperature increased, the captive females became active and attempted to break out of their wooden holding cages. Blood samples were collected from four of the six animals prior to 12 February, during the temperature crisis (12 to 18 February), and during the postcrisis after the animals were removed from the barn and transplanted to natural dens with cooler environments.

Blood samples were drawn with the use of a Vacutainer tube (Beckton-Dickinson, Rutherford, New Jersey 07070, USA). A 10 to 15 ml sample was drawn from a femoral vessel from each adult and yearling, and a 2 to 5 ml sample was collected from a jugular vein from each cub. The blood was allowed to clot for 30 min to 2 hr at room temperature and then centrifuged for 10 min. Serum was transferred to a vial containing stabilizers for enzymes and glucose and shipped fresh to the Medical Laboratory Network (Ventura, California 93002, USA) for chemical analyses.

All serum chemistries were performed on American Monitor's Parallel (American Monitor Corporation, Indianapolis, Indiana 46268, USA), a high speed sequential, computerized analyzer with 30 channels. Monitrol I and II (Dade Division of American Hospital Supply Corporation, Miami, Florida 33100, USA) were used for quality control. The methods used to determine blood chemistries are described in bulletins prepared by the American Monitor Corporation, and details on the composition and preparation of reagents and reaction products pertinent to the methodology are available from the Medical Laboratory Network.

To calculate serum urea, the serum urea nitrogen values (SUN) were divided by 0.466 since nitrogen represents 46.6% by weight of the urea molecule. This conversion made it possible to compare our results with those of Nelson et al. (1984) who found that urea/creatinine ratios decreased to ≤ 10.0 during winter dormancy.

Statistical analyses included the calculation of means, standard errors and minimum and maximum values of serum chemistries for the appropriate groups relative to age, sex, season and locality (captive versus free-ranging). *T*-tests were performed by using the Proc GLM procedure (SAS Institute Inc., 1985) to determine whether chemistries were significantly ($P < 0.05$) different between sexes for each of the cub and yearling groups, and to determine if chemistries were significantly different between bears sampled during the winter of 1984 and those sampled by Matula (1976) during the non-winter months of 1972 to 1974.

The Bonferroni method for multiple comparisons was used to test for significant differences among mean levels of serum chemistries among the three age groups (cubs, yearlings, adults) of females and, among the three groups (captive females with cubs, captive females with yearlings and free-ranging females with cubs) of adult females. The Bonferroni method, with the overall alpha level set at 0.05, was chosen because it is conservative and is appropriate when sample sizes are equal or unequal (Neter et al., 1985).

RESULTS AND DISCUSSION

Sex differences

Of 24 serum chemistries, only SUN and creatinine were lower ($P < 0.05$), and iron was higher ($P < 0.05$) in male cubs compared to female cubs, whereas all chemistries of yearlings were similar ($P \geq 0.05$) between sexes (Table 1). Significant differences in SUN, creatinine and iron were not expected when female and male cubs were compared because there were no significant differences between sexes for chemistries from yearlings. Based on the yearling findings, it was concluded that the sex differences in cub data were not significant from a physiological standpoint. It was possible that iron stores were depleted in some mothers who nursed cubs, and this could lead to a lower concentration of iron in some cubs. Yearlings, on the other hand who had the opportunity to

TABLE 1. The mean and standard error of blood chemistry values for female (F) and male (M) cub and yearling black bears during winter dormancy (February to early April 1984) in Pennsylvania.

Component	Cubs				Yearlings			
	F (n = 17) ^a		M (n = 20) ^b		F (n = 10)		M (n = 4)	
	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE
Calcium (mg/dl)	10.3	0.1	10.2	0.1	9.8	0.1	9.5	0.3
Inorganic phosphorus (mg/dl)	7.7	0.2	8.3	0.4	3.4	0.2	4.5	0.6
Sodium (meq/liter)	136.5	1.2	138.0	0.9	136.6	0.6	135.3	2.7
Potassium (meq/liter)	5.5	0.1	5.4	0.1	4.3	0.1	4.3	0.1
Chloride (meq/liter)	101.8	0.6	102.4	0.4	98.1	0.7	98.0	5.6
Iron (μ g/dl)	271.4*	36.2	374.1*	30.9	135.0	9.0	129.0	12.2
Total bilirubin (mg/dl)	0.3	0.0	0.8	0.3	0.1	0.0	0.3	0.1
Glucose (mg/dl)	107.2	11.0	80.3	12.1	112.2	27.3	156.0	65.4
SUN (mg/dl) ^c	9.5*	1.0	6.4*	0.4	6.7	0.8	5.0	1.2
Creatinine (mg/dl)	0.7*	0.0	0.6*	0.1	2.6	0.1	2.4	0.4
SUN/creatinine ^c	12.8	0.9	14.1	2.8	3.8	1.4	2.3	0.8
Uric acid (mg/dl)	1.8	0.1	1.9	0.1	1.5	0.2	2.1	0.4
Cholesterol (mg/dl)	350.7	25.8	319.6	18.9	391.5	36.8	512.5	101.2
Triglycerides (mg/dl)	352.9	33.5	306.1	24.8	463.5	96.1	471.0	103.3
Total lipids (mg/dl)	1,201.8	79.0	1,080.8	48.0	1,448.7	201.1	1,610.0	229.5
Total protein (g/dl)	5.3	0.2	4.6	0.3	7.0	0.1	6.7	0.1
Albumin (g/dl)	2.7	0.1	2.3	0.2	4.1	0.1	4.0	0.1
Globulin (g/dl)	2.7	0.2	2.3	0.2	2.9	0.1	2.7	0.1
Albumin/globulin ratio	1.0	0.1	1.0	0.1	1.4	0.1	1.5	0.0
Alkaline phosphatase (IU/liter)	193.9	15.3	188.4	17.4	47.5	4.8	54.0	14.2
LDH (IU/liter) ^c	560.0	36.4	514.5	34.0	545.5	65.6	477.8	56.9
SGOT (IU/liter) ^c	85.5	5.1	107.0	10.3	62.9	8.4	54.3	8.1
SGPT (IU/liter) ^c	30.4	1.3	34.0	3.2	31.0	7.5	18.8	6.5
GGTP (IU/liter) ^c	16.6	2.2	12.7	1.6	11.3	0.8	13.5	2.9

^a Sample size was ≥ 16 for each component.

^b Sample size was ≥ 17 for each component.

^c SUN, serum urea nitrogen; LDH, lactic dehydrogenase; SGPT, serum glutamate pyruvic transaminase = alanine amino transferase (ALT); SGOT, glutamate oxaloacetate transaminase = aspartate amino transferase (AST); GGTP, γ -glutamyl transferase.

* Significant ($P < 0.05$) difference between sexes within age group.

feed for an entire year, would probably eliminate such differences between sexes. The lack of significant differences between sexes for most chemistries is in agreement with previous data for black bears in Pennsylvania. Matula et al. (1980) reported that a low serum calcium level in females compared to that of males was the only serum chemistry value that was significantly different between sexes; he attributed this difference to the demands of pregnancy and lactation.

Age differences

The mean values for 15 of 24 serum chemistries of female cubs differed ($P < 0.05$) from those of yearlings and adults or both (Table 2). Total protein, albumin and

creatinine levels were lower ($P < 0.05$) in cubs than in yearlings and adults. Chloride, alkaline phosphatase, potassium, inorganic phosphorus and SUN/creatinine were higher ($P < 0.05$) in cubs than in yearlings and adults. Calcium was highest also in cubs, but the differences were significant ($P < 0.05$) only between cubs and adults. These differences were expected because higher calcium values in cubs are indicative of rapid changes in bone development and dietary intake. The relatively low serum calcium in adults was attributed to high calcium demands associated with lactation during winter dormancy and a lack of dietary intake during this period. Our data confirm Brannon's (1985) report of decreases in alkaline phos-

TABLE 2. Mean blood chemistry values of cub (C), yearling (Y) and adult (A) free-ranging female black bears during winter dormancy (February to early April 1984) in Pennsylvania.

Component	C (n = 17)	Y (n = 10)	A (n = 14)	Significance ^a
Calcium (mg/dl)	10.3	9.8	8.3	<u>CYA</u>
Inorganic phosphorus (mg/dl)	7.7*	3.4	4.6	<u>CAY</u>
Sodium (meq/liter)	136.5	136.6	138.8	<u>AYC</u>
Potassium (meq/liter)	5.5*	4.3	3.9	<u>CYA</u>
Chloride (meq/liter)	101.8*	98.1	97.9	<u>CYA</u>
Iron (μ g/dl)	271.4	135.0	201.9	<u>CAY</u>
Total bilirubin (mg/dl)	0.3	0.1	0.3	<u>CAY</u>
Glucose (mg/dl)	107.2	112.2	96.1	<u>YCA</u>
SUN (mg/dl) ^b	9.5	6.7	9.6	<u>ACY</u>
Creatinine (mg/dl)	0.7*	2.6	2.8	<u>AYC</u>
SUN/creatinine ^b	12.8*	3.8	3.4	<u>CYA</u>
Uric acid (mg/dl)	1.8	1.5	1.1	<u>CYA</u>
Cholesterol (mg/dl)	350.7	391.5	350.4	<u>YCA</u>
Triglycerides (mg/dl)	352.9	463.5	382.2	<u>YAC</u>
Total lipids (mg/dl)	1,201.8	1,448.7	1,274.2	<u>YAC</u>
Total protein (g/dl)	5.3*	7.0	7.2	<u>AYC</u>
Albumin (g/dl)	2.7*	4.1	3.6	<u>YAC</u>
Globulin (g/dl)	2.7	2.9	3.6	<u>AYC</u>
Albumin/globulin ratio	1.0	1.4	1.0	<u>YCA</u>
Alkaline phosphatase (IU/liter)	193.9*	47.5	18.8	<u>CYA</u>
LDH (IU/liter) ^b	560.0	545.5	345.5	<u>CYA</u>
SGOT (IU/liter) ^b	85.5	62.9	91.4	<u>ACY</u>
SGPT (IU/liter) ^b	30.4	31.0	28.1	<u>YCA</u>
GGTP (IU/liter) ^b	16.6	11.3	17.3	<u>ACY</u>

^a Letters with the same underline indicate mean values were not significantly different ($P \geq 0.05$).

^b See Table 1 for abbreviations.

* Mean values of cubs were higher or lower ($P < 0.05$) than those of both yearlings and adults.

phatase, phosphorus and calcium with age, and lower serum calcium in lactating females.

In the present study, the mean urea/creatinine ratios of 5.5 and 4.6 for yearling females and males, respectively, and 6.3 for adult females were lower ($P < 0.05$) compared to those of cubs (29.0 for females and 22.8 for males). The marked differences in urea/creatinine ratios between cubs and adult females were highlighted by plotting the values for cubs and their respective mothers (Fig. 1). These results support the findings of Nelson et al. (1984) that adult bears, including lactating females, showed urea/creatinine values of ≤ 10.0 during winter dormancy. The ratio of blood urea to creatinine decreases from ≥ 20 in summer to ≤ 10 in winter (Nelson et al., 1984). This indicator of successful

adaptation to food or water has been found in bears in captivity and in wild bears from Colorado, Maine, Pennsylvania and Minnesota (Ensrud et al., 1986). Development of the low urea to creatinine ratio is life-saving; bears who can not do so become uremic and die (Nelson, 1980).

Our findings extend the observations reported by Nelson et al. (1984) by demonstrating that nursing cubs have urea/creatinine values similar to other growing mammals. Unlike the adult females, the cubs were not in a biochemical state of hibernation, indicating that adaptive mechanisms for hibernation apparently were not yet developed in these cubs.

Seasonal effects

A comparison of blood from bears during the winter and non-winter indicated

that calcium, inorganic phosphorus, potassium, cholesterol and alkaline phosphatase were higher ($P < 0.05$) in young cubs in winter than older cubs in late April to December. This comparison reaffirmed that these serum chemistries were inversely related to age, and that the higher calcium, inorganic phosphorus and alkaline phosphatase levels in cubs are associated with rapid growth. Sodium, glucose, SUN, total protein, globulin, albumin/globulin ratio, lactic dehydrogenase (LDH) and serum glutamate oxaloacetate transaminase (SGOT) were markedly lower in cubs in winter than during late April to December. We attributed this finding, in part, to the increase in activity during the season when cubs have left their dens.

The mean cholesterol levels of cubs and yearlings during winter dormancy ranged from 319.6 to 512.5 mg/dl. These levels were significantly ($P < 0.05$) higher than the means of 246.4 and 294.5 for cubs and yearlings, respectively during the non-winter (Matula et al., 1980). Increased cholesterol in cubs and yearlings in winter support the report by Nelson et al. (1973) that cholesterol increases during hibernation. The elevation in cholesterol in cubs during winter dormancy probably reflected cholesterol intake from the milk of bears, a species that has the second highest concentration of lipids reported in mammals (Nelson et al., 1973).

Serum chemistries of black bears seem to be relatively stable during winter denning, when bears are without food and water and do not urinate or defecate for several months (Nelson, 1973). This remarkable response clearly shows that black bears are resistant to the effects of no food or water while existing at a near normal body temperature. There are no other mammals that have been reported to show such a resistance to the effects of extreme metabolic stresses imposed by the lack of food and water. Given the observed stability of serum chemistries during winter denning, we believe that abnormal blood chemistry values may indicate metabolic

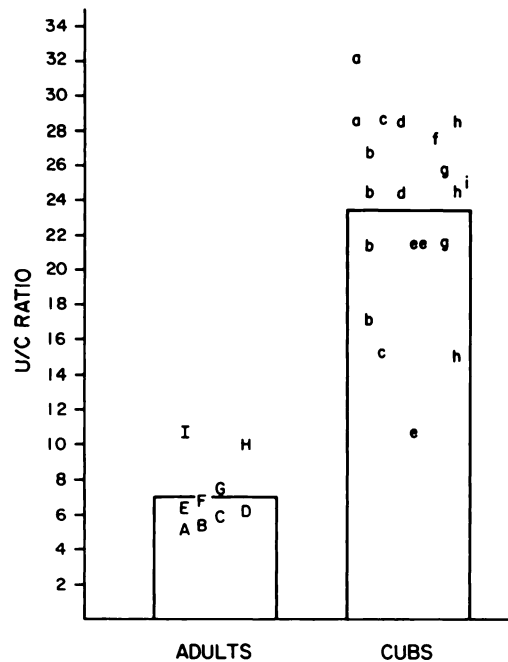


FIGURE 1. The urea/creatinine (U/C) ratios of nine adult female black bears and their cubs. Cubs from the same litter (litter size ranged from one to four) and their mother are represented by the same letter (lower case for cubs and capitalized for adults). The horizontal line at the top of each rectangle represents the mean U/C ratio for each group.

stresses that are not being controlled by the bear.

Captive versus free-ranging bears

Levels of gamma glutamyl transpeptidase (GGTP), sodium, calcium, chloride, total protein, albumin, SUN and SUN/creatinine were higher ($P < 0.05$) in captive adult females with cubs than their counterparts in the wild. Conversely, uric acid was the only serum chemistry value that was lower ($P < 0.05$) in the captive group than in one (wild adults with cubs) of the other groups (Table 3).

Three of four bears sampled before, during and after they were subjected to unseasonably warm winter temperatures in captivity showed a decrease in glucose levels during the crisis. Glucose levels varied from 120 to 227 mg/dl before the crisis and from 3 to 18 mg/dl during the crisis. These three animals also had SUN levels

TABLE 3. Mean blood chemistry values of captive and free-ranging adult female black bears with offspring, February to early April 1984 in Pennsylvania.

Components	Captive (C) with cubs (n = 6)	Wild (W) with cubs (n = 14)	Wild (Y) with yearling (n = 6)	Significance ^a
Calcium (mg/dl)	10.3	8.3	9.0	<u>CYW</u>
Inorganic phosphorus (mg/dl)	4.3	4.6	3.2	<u>CWY</u>
Sodium (meq/liter)	149.3	138.8	135.5	<u>CWY</u>
Potassium (meq/liter)	3.9	3.9	4.1	<u>YCW</u>
Chloride (meq/liter)	106.7	97.9	98.8	<u>CYW</u>
Iron (μ g/dl)	223.5	201.9	151.2	<u>CWY</u>
Total bilirubin (mg/dl)	0.1	0.3	0.1	<u>WYC</u>
Glucose (mg/dl)	144.5	96.1	146.8	<u>YCW</u>
SUN (mg/dl) ^b	18.0	9.6	5.7	<u>CWY</u>
Creatinine (mg/dl)	3.1	2.8	3.2	<u>YCW</u>
SUN/creatinine ^b	5.5	3.4	2.0	<u>CWY</u>
Uric acid (mg/dl)	0.8	1.1	1.5	<u>YWC</u>
Cholesterol (mg/dl)	400.2	350.4	460.8	<u>YCW</u>
Triglycerides (mg/dl)	349.2	382.2	317.8	<u>WCY</u>
Total lipids (mg/dl)	1,297.0	1,274.2	1,404.8	<u>YCW</u>
Total protein (g/dl)	9.1	7.2	7.5	<u>CYW</u>
Albumin (g/dl)	5.0	3.6	4.3	<u>CYW</u>
Globulin (g/dl)	4.0	3.6	3.2	<u>CWY</u>
Albumin/globulin ratio	1.2	1.0	1.3	<u>YCW</u>
Alkaline phosphatase (IU/liter)	17.8	18.8	20.0	<u>YWC</u>
LDH (IU/liter) ^b	470.2	345.5	417.2	<u>CYW</u>
SGOT (IU/liter) ^b	28.2	91.4	78.3	<u>WYC</u>
SGPT (IU/liter) ^b	27.3	28.1	22.3	<u>WCY</u>
GGTP (IU/liter) ^b	31.5	17.3	16.8	<u>CWY</u>

^a Letters with the same underline indicate mean values were not significantly different ($P > 0.05$).

^b See Table 1 for abbreviations.

varying from 1 to 14 mg/dl during the crisis and from 12 to 71 mg/dl after the crisis. In addition, SGOT and serum glutamate pyruvic transaminase (SGPT) increased from the precrisis to crisis period. Two of the three animals with marked changes in blood chemistries were housed on the upper floor of the barn; these bears also showed the greatest increase in activity (restlessness and escape behavior). We concluded that the changes in the chemistry profiles of these captive bears were a result of stress due to high temperatures and close association of several bears in relatively restricted quarters.

Levels of sodium and total protein in serum of the captive females were higher than expected (Matula et al., 1980), indicating that all four animals may have been dehydrated during the February crisis.

Furthermore, urea/creatinine increased in two of the bears, and creatinine appeared abnormally high in all four. Thus, the increase in concentrations of creatinine, sodium, total protein and urea/creatinine support the possibility of dehydration. Apparently, the water of metabolism from fat combustion, which is usually sufficient to meet water requirements (Nelson, 1973), was insufficient to supply requirements for temperature regulation during the crisis. The stimulus to maintain normal temperature and the developing state of dehydration may be factors causing some bears to leave their dens during warm winter periods.

CONCLUSIONS

Variation in levels of serum chemistries of black bears was greater due to age than

to sex. Only three of 24 serum chemistry values were significantly different between female and male cubs, whereas none of the chemistry values of yearlings were significantly different between sexes. Serum levels of alkaline phosphatase, inorganic phosphorus and calcium were significantly higher in cubs than in adults, which is indicative of rapid changes in bone development in cubs. The nursing cubs, unlike the adult females, were not in a biochemical state of hibernation as indicated by the higher urea/creatinine ratio (>20) in cubs compared to those of adults (<10). This finding indicated that adaptive mechanisms for winter dormancy were not yet developed in cubs.

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