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EFFECTS OF SEX, AGE, CAPTURING METHOD, AND SEASON ON SERUM CHEMISTRY VALUES OF BROWN BEARS IN CROATIA

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ABSTRACT: Sixty seven serum samples collected from 43 European brown bears (*Ursus arctos*) from Croatia were tested for ≤31 serum chemistry parameters. Results were grouped and compared by bears origin (method of capture), sex, age, mass, and season sampled. Greatest differences were found between captive and free-living bears, and minor differences were found when sex, age, mass, or season of sampling were compared. Creatine kinase was significantly different among three categories of bears with the highest mean value of 924 IU/l in snare captured free-living bears compared to 67.8 IU/l in captive ones. Results of these tests provide reference values for European brown bears.

Key words: European brown bear, serum chemistry, survey, *Ursus arctos*.

INTRODUCTION

European brown bears (*Ursus arctos*) reached their lowest numbers and distributions in Europe in recent decades. They are fragmented into approximately 16 isolated populations (Servheen, 1990). Present attempts to enhance bear numbers and range depend on complete understanding of their biology including physiological values. Serum biochemistry data were published for American black bears (*Ursus americanus*) (Hock, 1966; Nelson et al., 1973, 1984; Matula et al., 1980; Schroeder, 1987; Hellgren et al., 1993), for American brown bears (*U. arctos*) (Brannon, 1985; Nelson et al., 1983). Nelson et al. (1973, 1983) reported serum biochemistry values of polar bears (*Ursus maritimus*). Data on European brown bear serum chemistry were published only by Jamnicky et al. (1987) and Hissa et al. (1994). Approximately 400 brown bears are believed to inhabit Croatia (Huber and Morić, 1989; Huber and Frković, 1993). They are part of the Dinara Mountains population which is the largest in southwestern Europe and is the source for reintroductions to other countries. The objectives of this study were to (1) determine reference values for serum chemistry parameters for European brown bears in Croatia, (2) determine if there were differences due to sex, age, mass, origin, method of capture or season of sampling, and (3) find differences compared to other bear populations and species.

MATERIALS AND METHODS

Blood samples were collected from 23 male and 20 female European brown bears. Twenty-six (18 males and eight females) were free-living and 17 (five males and 12 females) were captive. Free-living bears were sampled following capture by spring-activated foot snares (Huber et al., 1996) for radio-tagging in Croatia; nine in the Lika region (44°55′N, 15°39′E) and 17 in the Gorski kotar region (45°27′N, 14°38′E) (Huber and Roth, 1993). Captive bears were from the Zagreb Zoo (45°20′N, 16°02′E). All bears appeared clinically healthy at the time of sampling as determined by temperature, pulse rate and respiration rate measurements, and external veterinary inspection. Only 6 free-living bears had fresh skin lesions: 3 on heads due to bites by other bears during mating season and 3 on feet due to the capture cable. Age of free-living bears was estimated by counting cementum layers around the root of the extracted rudimentary first premolar tooth (Stoneberg and Jonkel, 1966). Age of captive bears was known from the Zoo records. Mean age of bears was 5.2 years (SD = 4.0, range 0.3 to 14 yr) comprising 18 subadults (≤3 yr) and 27 adults (>3 yr). According to the mass 28 bears were ≤100 kg (mean = 57 ± 32 kg) and 22 were >100 kg (mean = 138 ± 36 kg). Thirty-seven bears were tested in winter and spring (1 January through 30 June) and 12 in summer and fall (1 July through 31 December). No samples have been taken from hibernating

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bears. Results obtained from sera of bears which were sampled as subadults and again as adults (n = 2), with mass ≤ and >100 kg (n = 7), or in different seasons (n = 6) were used for calculations in each of the respective groups. For all other calculations, multiple samples from the same individual were averaged and then pooled with other data.

Blood samples were taken from the femoral vein or artery during chemical immobilization with ketamine hydrochloride (11 mg/kg, Ketalar, Parke-Davis, Berlin, Germany) and xylazine hydrochloride (6 mg/kg, Rompun, Bayer, Leverkusen, Germany). Drugs have been administered by CO_2 powered immobilizing gun from the 10 m distance to the snared free-living bears and to caged captive bears. After blood clotted serum was separated (1,200 G, 10 min) within 12 hr and stored at −20 C.

Serum parameters were analyzed in the Clinic for Internal Diseases (Veterinary Faculty, University of Zagreb, Croatia). Up to 31 serum chemistry values were determined from each sample (Table 1). Serum analysis was done on an automatic analyzer (Technicon RA 1000 analyzer, Technicon Instruments Corporation, Tarrytown, New York, USA), and by electrophoresis (Cellogel Electrophoresis, Chemetron, Milan, Italy). Reagents for all analyses including bovine (low and high) reference value serum were obtained from Randox Laboratories (Ardmore, Antrim, UK). Enzyme assays were done at 25 C, and alkaline phosphatase (AP) activity was measured at pH 10.5.

Means and their standard deviations (SD) were calculated for each parameter. Any value that differed from the mean >2.5 SD was classified as an extreme variation and excluded (n = 30 values for individual parameters) from further calculations (Werner and Marsh, 1975). The mean, SD and standard error (SE) for each parameter were then recalculated after removal of outliers. The t-test (Burington and May, 1958) was used for statistical comparisons of results for different groups of bears; values of $P < 0.05$ were considered as statistically significant.

RESULTS

Mean and range values for 31 tested serum biochemistry parameters for chemically immobilized European brown bears are shown in Table 1. Large SD values for a number of serum chemistry values indicate individual variations.

For 10 of 31 serum parameters, one or more of five bear categories compared showed significant differences. Creatine kinase (CK) was significantly different among three compared categories and the other nine parameters: total bilirubin (TB), blood urea nitrogen (BUN), iron (Fe), unsaturated iron binding capacity (UIBC), potassium (K), aspartate amino transferase (AST), alanine amino transferase (AST), alpha 1 (α1), and gama (γ), differed among one category each. The greatest number of serum parameters (n = 8) with significant differences were found between captive and free-living bears: TB was 7.83 versus 15.8 μmol/l, BUN was 3.67 versus 7.62 mmol/l, Fe was 36.0 versus 23.6 μmol/l, UIBC was 22.6 versus 39.0 μmol/l, AST was 45.8 versus 150 IU/l, ALT was 17.1 versus 24.8 IU/l, CK was 67.8 versus 924 IU/l, and α1 was 3.62 versus 6.05%, respectively. One parameter was different when sex, mass, and age of bears, or season were compared: CK was 618 IU/l in males and 212 IU/l in females ($P < 0.054$), and 139 IU/l in bears ≤100 kg and 577 IU/l in bears >100 kg ($P < 0.015$), γ were 18.7% in subadults and 14.7% in adults ($P < 0.035$), and K was 4.42 mmol/l in winter–spring and 4.90 mmol/l in summer-fall season ($P < 0.039$). Bears with fresh skin lesions (n = 6) at the time of capture had higher CK values compared to other free-living and snare captured bears (2,330 versus 924 IU/l, respectively, $P < 0.09$).

DISCUSSION

Total bilirubin values described by Brannon (1985) for American brown bears (1.71 μmol/l) and by Matula et al. (1980) for American black bears (3.42 μmol/l) were below our mean values but within the range we found. Matula et al. (1980) found significantly higher TB values in subadult black bears compared to adults ($P < 0.05$). Young European brown bears from Croatia also had higher TB than adults but the difference of 3.8 μmol/l was not significant ($P < 0.19$). In our study TB values were significantly ($P < 0.005$) lower in captive compared to free-living bears which was probably due to better physical

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TABLE 1. Serum chemistry values for European brown bears from Croatia.

Serum parameter	All bears						Bear categories (shown are differences by $P < 0.05$)				
	Unit	*n*	Mean	Min.	Max.	SD	SE	Captive/ free-living	Male/ female	Subadult/ adult	≤100 kg/ >100 kg	Winter–spring/ summer–fall
Total bilirubin	µmol/l	47	12.4	0.20	41.1	9.59	1.40	−0.005[a]				
Total protein	g/l	33	71.1	54.9	87.8	7.96	1.39					
Albumin	g/l	28	36.2	20.2	48.2	6.52	1.23					
Globulin	g/l	27	35.8	22.2	51.8	8.01	1.54					
Creatinine	µmol/l	47	97.3	52.0	204	31.9	4.65					
Blood urea nitrogen	mmol/l	44	5.74	1.00	12.7	2.93	0.44	−0.001				
Glucose	mmol/l	45	7.30	1.57	14.9	3.00	0.45					
Iron	µmol/l	38	29.2	9.20	62.1	13.4	2.17	0.004				
Unstaturated iron binding capacity	µmol/l	29	28.8	2.30	64.7	17.0	3.15	0.013				
Total iron binding capacity	µmol/l	39	58.0	29.4	81.5	10.8	1.73					
Total cholesterol	mmol/l	22	7.02	5.32	12.1	1.58	0.34					
Triglycerides	mmol/l	22	4.09	1.90	8.16	1.48	0.32					
Potassium	mmol/l	11	4.51	3.90	5.20	0.31	0.09					−0.039
Sodium	mmol/l	12	143	133	155	6.46	1.86					
Chloride	mmol/l	7	108	103	120	5.94	2.25					
Calcium	mmol/l	13	2.16	1.68	2.89	0.27	0.07					
Anorganic phosphorus	mmol/l	14	1.82	0.65	3.03	0.56	0.15					
Magnesium	mmol/l	7	0.89	0.86	0.95	0.03	0.01					
Aspartate amino transferase	IU/l	45	101	19.0	480	101	15.1	−0.001				
Alanine amino transferase	IU/l	44	20.9	1.00	86.0	12.1	1.82	−0.049				
Cholinesterase	IU/l	4	970	750	1,134	162	81.1					
Creatine kinase	IU/l	22	342	32.0	1,220	448	95.7	−0.001	0.054		−0.015	
Alkaline phosphatase	IU/l	40	43.6	11.0	100	21.8	3.45					
Gamma glutamyl transferase	IU/l	46	13.3	1.00	27.0	5.54	0.82					
Amylase	IU/l	8	22.6	13.0	37.0	8.99	3.18					
Lactate dehydrogenase	IU/l	28	987	11.0	2,493	662	125					
Albumin	%	27	51.0	37.3	68.6	8.07	1.55					
Alpha 1	%	27	4.76	1.70	10.7	2.19	0.42	−0.003				
Alpha 2	%	27	10.4	3.10	23.4	4.49	0.86					
Beta	%	27	15.8	6.80	25.6	4.35	0.84					
Gamma	%	28	16.3	7.30	25.0	4.88	0.92			0.035		

[a] Minus (−) in front of *P* value means that the first mentioned bear category had lower average.

condition in captivity or myoglobin breakdown from muscle damage associated with snare capture of free-living bears.

Values for BUN were similar to those published by Brannon (1985) for American brown bears, but were >1 SD higher than what Hellgren et al. (1993), and Matula et al. (1980) published for American black bears. We found highly significant ($P < 0.001$) difference between captive and free-living bears. In contrast, Nelson et al. (1983) found much higher BUN content in captive (4.01 mmol/l) vs. various categories of wild (1.18 to 2.86 mmol/l) polar bears. These differences may be related to amount of protein in the diet as their metabolism in mammals results in urea excretion. Apparently the brown bears in Zagreb Zoo had less protein in the diet containing mostly bread and fruits than wild bears found in their natural habitat in Croatia (Cicnjak et al., 1987).

The ratio of BUN and Creatinine (CR) in our study was 0.059, which is similar to 0.075 reported by Brannon (1985) for American brown bears, but higher than 0.013 to 0.038 in polar bears (Nelson et al., 1983) and 0.011 (summer) to 0.018 (winter) in American black bears (Nelson et al., 1984). Low winter BUN and high CR was described as a biological indicator of bear hibernation by Nelson et al. (1984).

Captive bears had significantly higher Fe content ($P < 0.04$) than free-living ones, while free-living bears exhibited higher UIBC ($P < 0.013$) compared to the captive ones. These differences may be related to licking of iron bars of cages in which most of the captive bears were kept during sampling.

Both AST and ALT ranges varied greatly in our bears as well as in American brown and black bears (Brannon, 1985; Schroeder, 1987), respectively. The method of physical restraint of the bear is mostly responsible for these variations. Schroeder (1987) found significantly ($P < 0.01$) elevated values for both enzymes for snare vs. culvert-trap-captured bears. We determined significantly lower AST and ALT for captive vs. free-living (snare captured) bears: $P < 0.001$ and $P < 0.05$, respectively. This may suggest AST and ALT are good indicators of muscle tissue damage which occurs due to bears effort to escape from the snare. Muscle damage probably accounts for the equally dramatic difference we found for CK in captive and free-living bears ($P < 0.001$). However, the bears captured by foot snares did not die or have lasting damage as confirmed by radio-telemetry tracking (Huber and Roth, 1993). Higher CK in males and >100 kg bears compared to females and bears ≤100 kg bears ($P < 0.05$ and $P < 0.015$, respectively) may be related to the general larger mass of muscles in males and bigger bears, as well as to their greater exertion during capture.

In conclusion, results of this study of serum biochemistry parameters provides reference values for European brown bears. Many values exhibited a wide range of variation caused by individual and group differences. Differences, some statistically significant, between European brown bears and published values for American brown bears, American black bears and polar bears were found. No evidence of disease unrelated to capture and intraspecific bites was found based on serum biochemistry data in any bears in our study, including serological tests (Madić et al., 1993, Modrić and Huber, 1993).

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