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SERUM BIOCHEMISTRY OF CAPTIVE AND FREE-RANGING GRAY WOLVES (*CANIS LUPUS*)

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Abstract: Normal serum biochemistry values are frequently obtained from studies of captive sedentary (zoo) or free-ranging (wild) animals. It is frequently assumed that values obtained from these two populations are directly referable to each other. We tested this assumption using 20 captive gray wolves (*Canis lupus*) in Minnesota, USA, and 11 free-ranging gray wolves in Alaska, USA. Free-ranging wolves had significantly ($P < 0.05$) lower sodium, chloride, and creatinine concentrations and significantly higher potassium and blood urea nitrogen (BUN) concentrations; BUN to creatinine ratios; and alanine aminotransferase, aspartate aminotransferase, and creatine kinase activities relative to captive wolves. Corticosteroid-induced alkaline phosphatase activity (a marker of stress in domestic dogs) was detected in 3 of 11 free-ranging wolves and in 0 of 20 captive wolves ($P = 0.037$). This study provides clear evidence that serum biochemical differences can exist between captive and free-ranging populations of one species. Accordingly, evaluation of the health status of an animal should incorporate an understanding of the potential confounding effect that nutrition, activity level, and environmental stress could have on the factor(s) being measured.
Key words: Gray wolf, exercise, activity level, corticosteroid-induced alkaline phosphatase.

INTRODUCTION

Normal serum biochemistry values are frequently obtained from studies of captive sedentary (zoo) or free-ranging (wild) animals. Direct comparisons of serum biochemical values obtained from captive and free-ranging members of the same species are uncommon,[27,29] and it is frequently assumed that values obtained from these two populations are directly referable to each other. This assumes that nutrition, activity level, sex, age, seasonal variation, reproductive status, environmental stress, and capture stress have minimal impact on serum biochemical constituents. Although many of these factors can be measured and controlled for when interpreting serum biochemical parameters, the effects of nutrition, activity level, and environmental stress are more difficult to predict. For example, to facilitate anesthetic safety, blood samples are usually obtained from captive animals after a controlled period of feed withdrawal, whereas the nutritional history of free-ranging animals is often unknown. Captive animals also have a much lower activity level than free-ranging animals, particularly for those species that travel large distances in search of food. Moreover, the availability of food and stability of the environment are more constant for captive animals than free-ranging animals. Therefore, we hypothesized that physiologically significant differences would exist in serum biochemical parameters between captive and free-ranging animals. We tested this hypothesis in gray wolves (*Canis lupus*), the archetypical endurance athlete. This allowed us to examine the potential effects that nutrition, activity level, and environmental stress could exert on serum biochemical parameters. We also examined whether the corticosteroid-induced alkaline phosphatase (CIALP) isoenzyme was present in wolf serum, as in dog serum,[7,8,10,21] because CIALP could be potentially useful as a biological marker for environmental stress in wolf populations.

MATERIALS AND METHODS

Study population

We examined 20 captive gray wolves (9 female, 11 male; aged 2–14 yr; mean body weight, 44.3 ± 8.9 kg) in January and 11 free-ranging gray wolves (2 female, 9 male; aged 1–6 yr; mean body weight, 42.3 ± 5.6 kg) in March in accordance with institutional guidelines. The captive wolves were housed in enclosures < 0.01 km² in east-central Minnesota (latitude 45°, longitude 93°), and had been kept in captivity for at least 2 yr. The free-ranging wolves inhabited the Yukon-Charley Rivers National Preserve in east-central Alaska (latitude 65°, longitude 143°) and had a territorial range of

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500–4,000 km^2. The density of free-ranging wolves in the study area was estimated at 5.4 wolves/1,000 km^2, which is low,[2] reflecting the scarcity of prey. Free-ranging wolves were examined in March because snow conditions, ambient temperature, and day length facilitated aerial tracking and capture of wolves.

Capture and restraint

Each captive wolf was cornered in its enclosure and administered tiletamine/zolazepam (Telazol, Fort Dodge Laboratories, Fort Dodge, Iowa 50501, USA) 5.0 ± 0.5 mg/kg i.m. by pole syringe. Once anesthetized, captive wolves were hand carried to a building for examination. All wolves were administered anesthetic within 10 min of commencing foot pursuit. This anesthetic agent was selected because of its proven safety and efficacy in gray wolves[9,31] and because it does not cause physiologically significant alterations in acid–base status and serum electrocyte concentrations.[30]

Free-ranging wolves were captured as part of an existing National Park Service wolf ecology and demography study. The location of each free-ranging wolf was initially determined by snow-tracking or radiotelemetry to locate previously radiocollared pack members. Once observed from a fixed-wing aircraft, the location of each wolf was relayed by radio to a nearby helicopter. A helicopter crew member then darted the wolf at close range using a Cap-chur gun (Palmer Chemical and Equipment Co., Douglasville, Georgia 30133, USA) and 500 mg of tiletamine/zolazepam in a 3-ml dart, equivalent to 12.1 ± 0.5 mg combined tiletamine/zolazepam per kg body weight. A higher dosage of anesthetic agent was required for effective immobilization of free-ranging wolves, compared to captive wolves,[31] but the agent does not alter the concentration of serum biochemical constituents in wild canids.[30] Each free-ranging wolf was subsequently observed from the fixed-wing aircraft until the anesthetic agent had taken effect, at which time the wolf was transported by helicopter to the closest suitable fixed-wing landing site for examination. Most wolves were administered the anesthetic agent within 3–5 min of commencing helicopter pursuit. Recorded induction times ranged from 2 to 10 min, and averaged 5 min for wolves immobilized with one dart. Wolves requiring more than one dart for immobilization had longer pursuit and induction times. Darts with failing or weak internal charges were usually responsible for those captures requiring multiple darts.

Blood sampling and analysis

Blood samples were obtained from the cephalic vein and immediately transferred to 10-ml silicone-coated glass tubes (Vacutainer, Beckton-Dickinson, Rutherford, New Jersey 07070, USA) and placed on ice. Serum was separated within 6 hr and stored at below −20°C for no more than 2 wk before being thawed and automatically analyzed (Hitachi 911, Boehringer Mannheim Corporation, Indianapolis, Indiana 46250, USA). Serum CIALP isoenzyme activity was determined using levamisole inhibition, as adapted for the automatic analyzer.[8] The levamisole inhibition assay for canine CIALP is sensitive and accurate.[8] The serum sample was then refrozen and stored at −70°C before being analyzed for serum total and free thyroxine (T4) and 3,5,3′-triiodothyronine (T3) concentrations (Animal Health Diagnostic Laboratory, Michigan State University, Lansing, Michigan 48909, USA), using a radioimmunoassay procedure.[17] Serum thyrotropin-stimulating hormone (TSH) concentration was determined using a rabbit antibody specific for canine TSH and ^{125}I-human TSH as a competitive antigen.[16]

Statistical analysis

Data were tested for normality by calculating the Shapiro–Wilk statistic and variables with a nonnormal distribution or unequal variances were log transformed before two-way analysis of variance was performed, with the two main factors being group (captive or free-ranging) and sex (male or female). When the Shapiro–Wilk statistic indicated that the transformation procedure failed to produce an approximately normal distribution and equal variances, data were compared using nonparametric methods (Kruskal–Wallis test). Categoric data were compared by the Fisher's exact test. An alpha level of 0.05 was used for statistical analysis.

RESULTS

No significant effect was found of sex or interaction between sex and group on any serum biochemical parameter, so data for male and female wolves was pooled for statistical analysis. Because of the small number of females (n = 2) in the free-ranging population, the study had low power to detect an effect of sex on serum biochemical values.

Free-ranging wolves had significantly lower serum sodium, chloride, and creatinine concentrations and significantly higher potassium and blood urea nitrogen (BUN) concentrations; BUN to creatinine ratios; and serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatine kinase activities than did captive wolves (Table 1).

Table 1. Mean serum biochemical values for captive and free-ranging gray wolves. All values are concentrations, except alkaline phosphatase, corticosteroid-induced alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and creatine kinase, which represent activities. Serum total and free thyroxine, 3,5,3′-triiodothyronine, and serum thyrotropin-stimulating hormone concentrations are also reported.[a]

Factor	Captive (n = 20)	Free-ranging (n = 11)	Probability
Total CO_2 (mEq/L)	19.0 ± 0.9	20.2 ± 3.3	0.28
Sodium (mEq/L)	150.4 ± 3.6	147.3 ± 2.0	0.0219
Potassium (mEq/L)	4.4 ± 0.4	4.9 ± 0.3	0.002
Chloride (mEq/L)	118.6 ± 3.6	111.0 ± 2.3	<0.0001
Calcium (mg/dl)	9.5 ± 0.5	9.4 ± 0.7	0.81
Phosphorus (mg/dl)	2.8 ± 0.4	3.6 ± 1.3	0.069
Glucose (mg/dl)	111 ± 13	92 ± 50	0.24
Creatinine (mg/dl)	1.24 ± 0.18	1.01 ± 0.10	0.001
BUN (mg/dl)	19.6 ± 7.6	46.2 ± 23.5	0.0011
BUN/creatinine ratio	16.3 ± 5.8	47.5 ± 22.2	0.0003
Bilirubin (mg/dl)	0.20 ± 0.04	0.24 ± 0.10	0.16
Cholesterol (mg/dl)	169 ± 31	168 ± 33	0.92
ALP (U/L)	19 ± 5	75 ± 87	0.0004
CIALP (U/L)	0 ± 0	17 ± 36	0.015
ALT (U/L)	58 ± 40	109 ± 50	0.0004
AST (U/L)	31 ± 9	123 ± 103	<0.0001
CK (U/L)	120 ± 31	364 ± 189	<0.0001
Total protein (g/dl)	5.99 ± 0.22	5.99 ± 0.43	0.97
Albumin (g/dl)	3.87 ± 0.21	4.00 ± 0.33	0.22
Total T4 (nM/L)	12.4 ± 6.7	15.4 ± 7.6	0.70
Total T3 (nM/L)	1.18 ± 0.13	1.10 ± 0.30	0.20
Free T4 (pM/L)	9.7 ± 3.6	11.5 ± 8.3	0.92
Free T3 (pM/L)	4.0 ± 4.0	4.6 ± 2.0	0.59
TSH (mU/L)	19.2 ± 8.0	21.4 ± 10.6	0.72

[a] Values are mean ± SD: BUN = blood urea nitrogen; ALP = alkaline phosphatase; CIALP = corticosteroid-induced ALP; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CK = creatine kinase; T4 = thyroxine; T3 = 3,5,3′-triiodothyronine; TSH = thyrotrophin-stimulating hormone.

Corticosteroid-induced ALP activity was detected in 3 of 11 free-ranging wolves and in 0 of 20 captive wolves (P = 0.037, two-tailed Fisher's exact test). The CIALP activities in the three free-ranging wolves were 20, 51, and 116 U/L. Health problems were not detected on physical examination of these three wolves.

One free-ranging wolf may have been hypothyroid, based on a high serum TSH concentration (>200 mU/L; range for all other wolves, 8–41 mU/L), a total serum T4 concentration of 8 nM/L (range for all other wolves, 6–29 nM/L), and a free serum T4 concentration of 6 pM/L (range for all other wolves, 4–22 pM/L). This wolf was 4 yr old, was probably the alpha male, weighed 53 kg, was phenotypically normal, and had a CIALP activity of 0 U/L. Data from this wolf were not included in the determination of mean ± SD values for the serum thyroid hormone concentrations of free-ranging wolves. No differences were found in serum thyroid hormone concentrations between captive and free-ranging wolves (Table 1), and T4 and T3 autoantibodies were not detected in any wolf.

DISCUSSION

Protection of endangered species often involves capture and relocation of free-ranging animals into underpopulated or depopulated areas. A recent notable example of such efforts is the successful reintroduction of the gray wolf into Yellowstone National Park. Although numerous factors influence the long-term success of reintroduction programs, an essential component is a healthy and physiologically normal animal. Assessment of health and physiologic status is often based on physical examination and laboratory tests, such as serum biochemical analysis. Serum biochemical values can be influenced by nutritional state[4,5,10,22,25,26] and level of exercise activity,[11,12,19] and this could account, at least in part, for the large number of physiologically significant differences in serum biochemical parameters that were found between these two wolf populations. However, because of the relatively small sample sizes, we do not advocate formulation of normal ranges for serum biochemical values in free-ranging and captive gray wolves from our data.

The most striking biochemical difference between free-ranging and captive wolves was the high BUN concentration in free-ranging wolves. We believed this finding reflected recent consumption of a high-protein meal by free-ranging wolves, as BUN is influenced by protein intake and renal function[4] whereas the serum creatinine concentration, which is influenced by renal function and skeletal muscle mass relative to glomerular filtration rate,[4] was significantly lower in free-ranging wolves than in captive wolves. Even though Alaskan gray wolves frequently have age-related renal cortical lesions of unknown etiology,[18] the elevated BUN and BUN to creatinine ratio in free-ranging wolves were more indicative of recent ingestion of a high-protein meal than the presence of renal disease. High BUN concentrations of the magnitude observed in free-ranging wolves are frequently observed in healthy dogs that have eaten a high-protein meal 4–18 hr previously,[4,10] whereas the BUN concentrations of captive wolves in the study reported here were similar to that reported previously for captive wolves.[3,23] Differences in BUN concentration have also been observed in different populations of white-tailed deer (*Odocoileus virginianus*),[25] pronghorn antelope (*Antilocapra americana*),[22] and bighorn sheep (*Ovis canadensis*)[5] grazing in different habitats. Therefore, the mean BUN concentration of a wild animal population may be used as a guide to protein intake.[26]

The lower serum sodium and chloride concentrations in free-ranging wolves may reflect a normal physiologic response, as decreased resting serum sodium and chloride concentrations have been observed in experimental studies involving gray wolves,[19] greyhound dogs,[11] and horses[12] undergoing prolonged endurance exercise training. The wolf has long been considered the archetypical endurance athlete, as its hunting behavior and social structure mandate travelling long distances in search of prey. Seasonal movements of up to 360 km have been observed in gray wolves following caribou migration and daily movements of up to 65 km have been observed in winter.[28] Depending upon the availability and type of prey, winter daytime activity studies indicate that wolves spend approximately 30% of each day travelling.[13] Based on an average travelling speed of 8.7 km/hr[14] and assuming that daytime activity reflects nighttime activity, gray wolves are suspected to travel an average of 63 km/day during winter in Alaska. The free-ranging wolves examined in this study were therefore presumed to have undergone many months of extreme endurance exercise activity. The reason that serum sodium concentration (and therefore serum osmolality) decreases with endurance exercise training is uncertain, but has been attributed to increasing the physiologic reserve against exercise-induced dehydration.[19] By lowering resting serum osmolality, endurance-trained wolves increase the degree of dehydration that they can sustain during exercise before dehydration-induced high serum osmolality decreases work performance.[19]

Our finding that serum thyroid hormone concentrations were similar in captive and free-ranging wolves was not surprising, as prolonged daily exercise has no effect on thyroid hormone concentrations in dogs.[1] Serum T4 concentrations for gray wolves were similar to those previously reported for captive animals[23]; however, compared to the normal laboratory values for dogs, gray wolves had a lower serum total T4 concentration (dog normal range, 15–50 nM/L) and a lower serum free T4 concentration (dog normal range, 12–33 pM/L).

The exercise activity associated with capture or free-ranging lifestyle may have induced mild skeletal muscle injury in free-ranging wolves, as physiologically mild but statistically significant increases in serum creatine kinase (CK) and AST activities were observed in this group (Table 1); however, the serum CK activity in free-ranging wolves was similar to that of Mexican wolves captured with nets and restrained with V-shaped poles.[3] Similar mild increases in CK and AST activities have also been observed in Alaskan sled dogs competing in a long-distance race[6]; the changes are considered to be physiologically unimportant. The significantly greater serum ALT, AST, and ALP activity in free-ranging wolves might be indicative of mild hepatocellular injury, although the lack of any difference in serum total bilirubin, glucose, cholesterol, and albumin concentrations between free-ranging and captive wolves suggests that liver function was only mildly impaired in free-ranging animals.

We believe this study is the first to document the presence of the CIALP isoenzyme in a species other than the domestic dog. Our finding that CIALP is present in gray wolves is consistent with current evolutionary theory that the domestic dog evolved from gray wolves 14,000–135,000 yr ago, and that the wolf is the canid most closely related to the domestic dog.[15,32] Alkaline phosphatase catalyzes the hydrolysis of phosphate esters at alkaline pH and is present in most organs of mammalian species.[21] Three isoenzymes of ALP have been identified in dog serum, liver, bone, and corticosteroid-induced ALP,[21] and presumably all three isoenzymes are present in wolf serum. Corticosteroid-induced ALP is produced in the canine liver by the portion of the hepatocyte membrane that composes the bile canaliculi.[20] The production of CIALP is induced by exposure to high concentrations of endogenous and exogenous corticosteroids.[20,21] Serum CIALP activity may therefore provide a useful index of environmental stress when applied to wolf populations or of general health when applied to individual wolves. Because the clearance of CIALP from canine blood is slow (the half-life of serum CIALP is approximately 72 hr),[7] the presence of CIALP in wolf serum could potentially indicate sustained physiologic stress, and not a transient response to capture. Serum CIALP concentration could potentially provide a more specific measure of stress than serum cortisol concentration in free-ranging wolves, and possibly be more sensitive and specific than fecal corticosterone concentration. It is of interest to note that CIALP was detected in a significantly greater proportion of free-ranging wolves (3 of 11) than in captive wolves (0 of 20). This study provides clear evidence that serum biochemical differences can exist between captive and free-ranging populations.

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