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Serum Biochemistry and Serum Cortisol Levels of Immobilized and Hunted Muskoxen (Ovibos moschatus) from Northern Canada

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ABSTRACT. Muskoxen (Ovibos moschatus) are Arctic-adapted ruminants native to the Arctic regions of Canada, Alaska, and Greenland. Little is known about the serum biochemistry and serum cortisol of this species, or the effects of chemical immobilization on serum biochemical parameters. This study aimed to describe blood chemistry parameters and cortisol levels in hunted, tame, and chemically immobilized muskoxen and to examine differences in blood chemistry parameters and levels of stress associated with different capture techniques. Serum was collected from 91 adult female muskoxen in northern Canada. For analysis, these muskoxen were classified into six groups, five of free-ranging muskoxen (10 animals shot from snowmobiles near Kugluktuk, Nunavut; 18 chemically immobilized from a helicopter near Kugluktuk; 8 chemically immobilized from a helicopter near Norman Wells, Northwest Territories; 17 shot from snowmobiles near Cambridge Bay, Nunavut; 33 chemically immobilized from a snowmobile near Kugluktuk) and one of tame muskoxen (five tame animals maintained on pasture near Saskatoon, Saskatchewan). All samples were analyzed for cortisol, and 26 serum biochemistry parameters were measured in serum collected from three of the six groups (n = 36). Comparison of four groups showed that serum cortisol levels of muskoxen chemically immobilized from a helicopter near Kugluktuk were significantly higher (p < 0.05) than those of muskoxen chemically immobilized from snowmobiles or shot. A comparison of serum biochemistry from the groups of muskoxen shot and immobilized near Kugluktuk found that serum sodium, creatinine, phosphorus, magnesium, and creatine kinase were significantly higher (p < 0.05) in hunted muskoxen than in chemically immobilized animals, while urea, glucose and gamma glutamyl transferase were significantly higher (p < 0.05) in immobilized muskoxen. Most serum biochemical parameters, however, were similar to those of captive muskoxen. This evidence of differences between hunted and immobilized muskoxen in several serum biochemistry parameters will contribute to further research on the effects of immobilization and other health assessments in this species.

Key words: muskoxen, Ovibos moschatus, serum biochemistry, chemical immobilization, stress

RÉSUMÉ. Le bœuf musqué (*Ovibos moschatus*) est un ruminant adapté à l'Arctique qui est natif des régions arctiques du Canada, de l'Alaska et du Groenland. On en sait peu à propos de la biochimie du sérum et du cortisol du sérum de cette espèce ou encore, à propos des effets de l'immobilisation chimique sur les paramètres biochimiques du sérum. Cette étude visait à décrire les paramètres de la chimie du sang et les taux de cortisol chez les bœufs musqués chassés, apprivoisés et chimiquement immobilisés, de même qu'à examiner les différences sur le plan des paramètres de la chimie du sang et des degrés de stress en fonction de diverses méthodes de capture. Du sérum a été prélevé auprès de 91 femelles adultes du nord du Canada. Aux fins de l'analyse, ces bœufs musqués ont été classés en six groupes, dont cinq des groupes étaient composés de bœufs musqués en liberté (10 des bœufs avaient été tirés depuis des motoneiges près de Kugluktuk, au Nunavut; 18 avaient été chimiquement immobilisés à partir d'un hélicoptère près de Kugluktuk; 8 avaient été tirés depuis des motoneiges près de Kugluktuk) et l'autre groupe était composé de bœufs musqués (5 bêtes évoluant dans des pâturages près de Saskatoon, en Saskatchewan). Dans tous les cas, le cortisol des échantillons a été analysé, puis 26 paramètres biochimiques du sérum ont été mesurés à partir du sérum recueilli chez trois des six groupes (n = 36). Les comparaisons établies pour quatre des groupes ont permis de constater que les

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taux de cortisol du sérum des bœufs musqués chimiquement immobilisés à partir d'un hélicoptère près de Kugluktuk étaient considérablement plus élevés (p < 0,05) que ceux des bœufs musqués qui avaient été chimiquement immobilisés depuis une motoneige ou qui avaient été tirés. La comparaison de la biochimie du sérum chez les bœufs musqués tirés et immobilisés près de Kugluktuk a permis de déceler que le sodium du sérum, la créatinine, le phosphore, le magnésium et la créatine kinase étaient considérablement plus élevés (p < 0,05) chez le bœuf musqué chassé que chez le bœuf musqué chimiquement immobilisé, tandis que l'urée, le glucose et la gamma-glutamyl-transférase étaient considérablement plus élevés (p < 0,05) chez le bœuf musqué immobilisé. Cependant, la plupart des paramètres biochimiques du sérum étaient semblables à ceux des bœufs musqués en captivité. Ces différences évidentes sur le plan de nombreux paramètres biochimiques du sérum entre le bœuf musqué chassé et le bœuf musqué immobilisé permettront d'approfondir les recherches sur les effets de l'immobilisation et d'autres évaluations de santé de cette espèce.

Mots clés : bœufs musqués, Ovibos moschatus, biochimie du sérum, immobilisation chimique, stress

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INTRODUCTION

Serum biochemistry is an important tool in the clinical evaluation of wild and domestic animals (Lepitzki and Woolf, 1991) and can provide indications of health and disease, nutritional status, and even habitat quality (Barnes et al., 2008). Different methods of capture may affect serum biochemical parameters in wildlife and may adversely affect the health, behaviour, and physiology of the subject, as well as the data gathered for research (Rietkerk et al., 1994; Marco et al., 1997; Kock et al., 1999; Marco and Lavin, 1999; Montane et al., 2002; Cattet et al., 2003, 2008). Therefore, serum biochemical parameters of wild captured animals have been widely reported (Rietkerk et al., 1994; Marco et al., 1997; Kock et al., 1999; Marco and Lavin, 1999; Montane et al., 2002; Cattet et al., 2003). Muskoxen (Ovibos moschatus) are Arctic-adapted ruminants that are native to the Arctic regions of Canada, Alaska, and Greenland (Nilssen et al., 1994). As these large, sedentary herbivores are found in remote locations, the capture and sampling of live, free-ranging animals are logistically challenging (Jingfors and Gunn, 1989). Information on serum biochemical parameters in muskoxen is limited (Glover and Haigh, 1984; Tedesco et al., 1991a, b), and there are no reports on the effects of chemical immobilization on their serum biochemistry.

The objectives of this study were to provide a description of blood chemistry parameters and serum cortisol levels in hunted, tame, and chemically immobilized muskoxen and to examine differences in blood chemistry parameters and levels of stress associated with different capture techniques. In this retrospective analysis of serum biochemical parameters from adult, female, free-ranging muskoxen either chemically immobilized by remote drug delivery systems or shot by local subsistence hunters, we report serum cortisol levels from six different groups of muskoxen and serum biochemical values from three of those groups. Although differences in processing and analysis of serum from some of the groups present challenges for drawing definite conclusions about the effects of immobilization on serum biochemistry in muskoxen, we hypothesized that physiological differences between hunted and chemically immobilized

muskoxen would be present. Serum biochemistry is an important tool in veterinary and wildlife medicine and is presented here because these data are useful in the clinical evaluation of wild muskoxen (Lepitzki and Woolf, 1991; Marco and Lavin, 1999; Barnes et al., 2008).

MATERIALS AND METHODS

Serum was collected from 86 free-ranging muskoxen chemically immobilized or shot in northern Canada and 5 tame muskoxen for analysis of biochemistry parameters and cortisol levels (Table 1). Muskoxen were retrospectively allocated to a particular group in this study depending on their location, capture method (chemical immobilization or shot), and date of sample collection. Each muskox was examined by a wildlife veterinarian conducting immobilizations, and none of the animals showed overt signs of disease or pre-existing injury at the time of sample collection. Body condition scores were not evaluated because it is difficult to assess body condition reliably and accurately in live muskoxen.

Study Groups

Group 1 (Kugluktuk-shot): Ten adult, female, freeranging, wild muskoxen were shot near Kugluktuk, Nunavut (formerly Coppermine, Northwest Territories, 67° 54' N, 116° 38' W) in November 1991. Before being shot, the muskoxen were pursued over open tundra by local hunters on snowmobiles. Actual chase times were not recorded but were estimated to be less than 30 min. Immediately after the animal's death, the jugular vein was cut. Blood was collected in sterile evacuated tubes (Vacutainer, Beckton-Dickinson, Rutherford, New Jersey) and placed in a padded pocket close to the hunter's body to prevent freezing. Within 2 to 12 hours of collection, clotted blood was centrifuged for 10 min. at 4000 rpm, and serum was removed and stored at -20°C for seven months until laboratory analysis could be performed at the Manitoba Agriculture Veterinary Diagnostic Services (MAVDS) laboratory in Winnipeg, Manitoba. Serum samples were analyzed for

| Group No. | Date | Location | n | Capture method | Type of approach | Method of blood collection | Serum analysis |
|-----------|----------------|---------------|----|------------------------|------------------|----------------------------|--|
| 1 | November 1991 | Kugluktuk | 10 | Shot | Snowmobile | Jugular cut-down | Biochemical |
| 2 | September 1991 | Kugluktuk | 18 | Chemically immobilized | Helicopter | Venipuncture | Biochemical parameters and cortisol |
| 3 | 2007/2008/2009 | Norman Wells | 8 | Chemically immobilized | Helicopter | Venipuncture | Biochemical parameters and cortisol |
| 4 | 1990/1991 | Saskatoon | 5 | Tame animals | Not required | Indwelling cannula | Cortisol |
| 5 | November 1991 | Cambridge Bay | 17 | Shot | Snowmobile | Jugular cut-down | Cortisol |
| 6 | March 1991 | Kugluktuk | 33 | Chemically immobilized | Snowmobile | Venipuncture | Cortisol |

TABLE 1. Date, location, capture method, blood collection methods, and parameters measured for serum analysis for six groups of muskoxen (*O. moschatus*) from northern Canada.

sodium, potassium, calcium, magnesium, phosphorus, urea, creatinine, glucose, total bilirubin, direct bilirubin, gamma glutamyl transferase (GGT), creatine kinase (CK), aspartate aminotransferase (AST), alkaline phosphotase (ALP), cholesterol, total protein, globulin, and albumin using a biochemistry analyzer (Dacos Wet Chemistry Analyzer, Coulter Electronics of Canada Ltd. [now Beckman Coulter Canada Inc., Mississauga, Ontario]), as well as for serum cortisol (TDx analyzer, Abbott Diagnostics, Abbott Park, Illinois).

Group 2 (Kugluktuk-helicopter immobilized): Eighteen adult, female, free-ranging, wild muskoxen located southwest of Kugluktuk, Nunavut, were chemically immobilized in September 1991 and captured for removal of radio collars. The muskoxen were located by radio tracking from a fixed-wing aircraft. A helicopter was used to approach the herd, and the radio-collared cows were immobilized by remote injection (Capchur Extra Long Range Projector, Palmer Capchur Equipment Co. Inc., Powder Springs, Georgia, and Distinject Model 60, Dist-Inject International, Basel, Switzerland) using a combination of carfentanil citrate (Wildnil, Wildlife Pharmaceuticals, Fort Collins, Colorado) at 0.01 mg/kg estimated body weight, and acepromazine (Atravet, Ayerst Laboratories [now Wyeth Animal Health, Guelph, Ontario]) at 0.05 mg/kg estimated body weight. Muskoxen were administered the immobilization drugs on average 4.24 min. after initial approach and subsequent helicopter pursuit. Naltrexone hydrochloride (Wildlife Pharmaceuticals, Fort Collins, Colorado) was given intramuscularly (100 mg naltrexone per 1 mg carfentanil) to reverse the effects of carfentanil. Average induction time was 5.86 min., and blood was collected within 5 to 30 min. after immobilization. Blood was collected into a sterile tube by venipuncture of the jugular vein and immediately chilled by placing the sample on ice until further processing. Within 2 to 12 hours of collection, blood was centrifuged for 10 min. at 4000 rpm, and serum was removed and stored at -20°C for one month until laboratory analysis. The blood was analyzed as in Group 1, except that total and direct bilirubin and cholesterol levels were not determined.

Group 3 (Norman Wells-helicopter immobilized): During November 2007, January 2008, and February 2009, a total of eight adult, female, free-ranging, wild muskoxen were chemically immobilized near Norman

Wells, Northwest Territories (65° 17' N, 126° 48' W). The muskoxen were located by helicopter in the boreal forest and immobilized by remote injection (Pneudart rifle, Model 389, Pneu-dart Inc., Williamsport, Pennsylvania) with carfentanil citrate at 0.01 mg/kg estimated body weight, and xylazine (Rompun, Bayer Healthcare, Toronto, Ontario) at 0.1 mg/kg estimated body weight. Muskoxen were administered the immobilization drugs on average 9.5 min. after initial approach and subsequent helicopter pursuit. Naltrexone hydrochloride was delivered intramuscularly with a hand syringe at a ratio of 125 mg naltrexone per 1 mg carfentanil following animal sampling. Average induction time was 12.72 min., and blood was collected between 5 and 30 min. following induction. After venipuncture of the jugular vein, blood was collected into a sterile tube, which was immediately placed in a padded pocket close to an attending person's body to prevent freezing. Within 2 to 12 hours of collection, the clotted blood was centrifuged for 10 min. at 4000 rpm, the serum was removed and stored at -20°C for 16 months (one muskoxen captured in 2007), 13 months (four muskoxen captured in 2008), or 5 months (three muskoxen captured in 2009) until laboratory analysis. Serum samples were analyzed as in Group 1, except that indirect bilirubin, glutamate dehydrogenase, sorbitol dehydrogenase, bicarbonate, and anion gap were measured, but ALP and cholesterol levels were not. Serum biochemistry parameters were analyzed using a biochemistry analyzer (Hitachi 912 Analyzer, Roche Diagnostics, Laval, Ouebec) and serum cortisol levels were determined (Immulite analyzer, Siemens Medical Solutions Diagnostics, Los Angeles, California) at Prairie Diagnostic Services Inc., Saskatoon, Saskatchewan.

In addition to the three groups of muskoxen described above, three other groups of muskoxen had blood collected and analyzed only for cortisol levels.

Group 4 (tame): A group of five tame, captive muskoxen was sampled between March 1990 and December 1991. Details of the care and maintenance of muskoxen, blood collection protocol, and serum cortisol analysis have been described (Tedesco, 1996). Blood samples were collected from the jugular vein. Within one hour, blood samples were centrifuged at 1200 g for 10 min., and the serum was removed and stored at -22°C. Serum cortisol was determined using a radioimmunoassay kit (Amerlex Cortisol

| Parameter (units) | Group 1 (n = 10) | Group 2 (n = 18) | Group 3 (n = 8) | Domestic cattle MAVDS ¹ | Domestic cattle, PDS ² (n = 23) | Captive muskoxen ³ $(n = 15)$ |
|----------------------|---------------------|---------------------|--------------------|---------------------------------------|---|--|
| CK (U/L) | 562.0-6319.0 | 138.0-8752.0 | 126.0-416.0 | 0.0-500.0 | 0.0-490.0 | 0-600 |
| AST (U/L) | 90.0-270.0 | 86.0-299.0 | 48.0-89.0 | 0.0-375.0 | 62-150 | 40-108 |
| Urea (mmol/L) | see Tables 3 and 4 | see Tables 3 and 4 | see Tables 3 and 4 | 2.5-9.6 | 3.7-8.7 | 7.5-22.7 |

TABLE 2. Ranges of serum creatine kinase (CK), aspartate aminotransferase (AST), and urea for hunted or chemically immobilized, free-ranging, wild muskoxen (*O. moschatus*) from groups 1 (Kugluktuk – shot), 2 (Kugluktuk – helicopter immobilized), and 3 (Norman Wells – helicopter immobilized), domestic cattle (*Bos taurus*), and captive muskoxen.

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² Prairie Diagnostic Services, Saskatoon, Saskatchewan.

³ Values taken from Tedesco et al. (1991).

RIA kit, Code IM.2021, Amersham International [now GE Healthcare Medical Diagnostics, Piscataway, New Jersey]) (Tedesco, 1996).

Group 5 (Cambridge Bay-shot): Serum was collected from 17 adult, female, free-ranging, wild muskoxen in November 1991 near Cambridge Bay, Nunavut (69° 54' N, 106° 38' W). Muskoxen were rounded up using snowmobiles and shot by local hunters during a research project on body condition. Chase and round-up times were not recorded but were estimated to be less than 30 min. Immediately after the animal's death, the jugular vein was cut and blood was collected in sterile tubes and placed in a padded pocket close to the hunter's body to prevent freezing. Within 2 to 12 hours of collection, the clotted blood was centrifuged for 10 min. at 4000 rpm, and serum was removed and stored at -20°C for three months until laboratory analysis. Serum was analyzed for cortisol using a TDx analyzer at the Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan.

Group 6 (Kugluktuk-snowmobile immobilized): In March 1991, 33 adult, female, free-ranging, wild muskoxen were chemically immobilized near Kugluktuk, Nunavut. Animals were located by local hunters using snowmobiles; chase times were not recorded. Groups of muskoxen gathered in a defensive formation and held this position for up to 10 min. before individual muskoxen were immobilized by remote injection with a combination of carfentanil citrate at 0.01 mg/kg estimated body weight and acepromazine at 0.05 mg/kg estimated body weight. Naltrexone hydrochloride was administered intramuscularly (100 mg naltrexone per 1 mg carfentanil) to reverse the effects of carfentanil. Average induction time was 5.0 min., and blood was collected between 5 and 30 min. after immobilization. Blood was collected into sterile tubes by venipuncture of the jugular vein and placed in a padded pocket close to an attending person's body to prevent freezing. Within 2 to 12 hours of collection, clotted blood was centrifuged for 10 min. at 4000 rpm, and serum was removed and stored at -20°C for one month until laboratory analysis. Serum was analyzed for cortisol using a TDx analyzer at MAVDS.

None of the methods used in this study for serum cortisol analysis (TDx analyzer, Immulite analyzer, RIA) were validated for ruminants, nor have the three methods been correlated with one another. The intra-assay coefficient of variation (CV) for the RIA ranged from 3.4% to 5.7% (Tedesco, 1996). Intra-assay CVs were not determined for Immulite or the TDx analyzers.

Reference values for serum CK, AST, and urea from domestic cattle (*Bos taurus*) from two clinical laboratories where muskox serum samples for this study were analyzed (Prairie Diagnostic Services Inc. and at the MAVDS laboratory) are included in Table 2 (unpubl. data).

Statistical Analysis

Because different methods of biochemical analysis and serum cortisol analysis were used, the biochemical parameters and serum cortisol levels were compared only between select groups of animals. All data were analyzed for normality using the Shapiro-Wilk test (Stata Version 10, StataCorp, College Station, Texas). Serum biochemistry parameters were not all normally distributed; therefore, non-parametric statistical tests were used. Serum biochemistry parameters for Group 1 (Kugluktuk-shot) and Group 2 (Kugluktuk-helicopter immobilized) were compared with a Wilcoxon rank sum test (SPSS Statistics 16.0, SPSS Inc., Chicago, Illinois). Serum cortisol levels for Groups 1 (Kugluktuk-shot), 2 (Kugluktuk-helicopter immobilized), 5 (Cambridge Bay-shot), and 6 (Kugluktuk-snowmobile immobilized) were compared with a Kruskal-Wallis test. Statistical significance was assigned when the probability of a type I error was equal to or less than 0.05. For statistical purposes, when a value was below the detection limit of the assay, the detection limit was the value recorded for the analysis (Norjavaara et al., 1996).

RESULTS

Hemolysis was encountered in several serum samples in each group. Of the 10 serum samples in Group 1 (Kugluktuk-shot), two were grossly hemolyzed and seven were slightly hemolyzed. Of the 18 samples in Group 2 (Kugluktuk-helicopter immobilized), two were grossly hemolyzed and 11 were slightly hemolyzed. Of the eight samples in Group 3 (Norman Wells-helicopter immobilized),

TABLE 3. Comparison of serum biochemistry parameters for free-ranging adult female muskoxen (O. moschatus) that were hunted (Group 1 – shot) or chemically immobilized (Group 2 – helicopter immobilized) near Kugluktuk, Nunavut. Bold type indicates significant differences between groups.

| | Muskox | en hunted, 1991 Group 1 | (n = 10) | Muskoxen immobilized, 1991 (n = 18) Group 2 | | | |
|--------------------------------|---------------------|----------------------------|--------------|--|--------|--------------|-----------------|
| Parameter (units) ¹ | Mean ± SD | Median | Range | Mean \pm SD | Median | Range | <i>p</i> -value |
| Sodium (mmol/L) | 144.3 ± 6.7^2 | 146.0 | 132.0-151.0 | 137.9 ± 2.6 | 137.0 | 134.0-143.0 | 0.004 |
| Potassium (mmol/L) | 6.9 ± 1.5 | 7.3 | 4.9-9.3 | 7.3 ± 1.4 | 6.3 | 5.2-10.2 | ns ³ |
| Na/K ratio | 21.0 ± 4.4 | 20.0 | 15.0-30.0 | 20.8 ± 4.25 | 22.0 | 13.0 - 27.0 | ns |
| Chloride (mmol/L) | 94.9 ± 3.8 | 94.5 | 88.0-100.0 | 96.5 ± 3.8 | 97.0 | 86.0-103.0 | ns |
| Bicarbonate (mmol/L) | NA^4 | NA | NA | NA | NA | NA | NA |
| Anion Gap (mmol/L) | NA | NA | NA | NA | NA | NA | NA |
| Urea (mmol/L) | 2.0 ± 1.0 | 2.3 | 1.8-3.6 | 5.2 ± 2.3 | 4.5 | 2.2 - 11.0 | < 0.0001 |
| Creatinine (mmol/L) | 325.0 ± 25.1 | 330.0 | 270.0-360.0 | 282.2 ± 29.8^2 | 290.0 | 210.0-310.0 | < 0.0001 |
| Glucose (mmol/L) | 4.8 ± 2.2 | 5.3 | 1.1 - 7.6 | 7.3 ± 1.6 | 7.5 | 4.9-10.4 | 0.028 |
| CK (U/L) | 2602 ± 1975 | 2294.0 | 562.0-6319.0 | 1527.8 ± 2172.7^2 | 662.0 | 138.0-8752.0 | 0.002 |
| AST (U/L) | 146.5 ± 54.1 | 141.0 | 90.0-270.0 | 140 ± 52.7^{2} | 125.0 | 86.0-299.0 | ns |
| GGT (U/L) | 97.0 ± 17.8 | 95.0 | 66.0-125.0 | 116.5 ± 21.9 | 115.5 | 86.0-168.0 | 0.001 |
| ALP (U/L) | 208.6 ± 252.7^2 | 92.5 | 39.0-806.0 | 154.4 ± 94.7^2 | 122.5 | 70.0-408.0 | ns |
| GLD (U/L) | NA | NA | NA | NA | NA | NA | NA |
| SDH (U/L) | NA | NA | NA | NA | NA | NA | NA |
| Total bilirubin (umol/L) | 4.8 ± 2.1 | 4.8 | 1.7-9.8 | NA | NA | NA | NA |
| Indirect bilirubin (umol/ | L) NA | NA | NA | NA | NA | NA | NA |
| Direct bilirubin (umol/L | $) 2.6 \pm 1.9$ | 1.9 | 0.5 - 5.4 | NA | NA | NA | NA |
| Cholesterol (units) | 2.5 ± 0.3 | 2.5 | 1.9-2.9 | NA | NA | NA | NA |
| Total protein (g/L) | 62.6 ± 22.5^2 | 68.5 | 49.0-81.0 | 68.9 ± 3.8^2 | 69.0 | 63.0-74.0 | ns |
| Albumin (g/L) | 37.1 ± 3.5^{1} | 37.4 | 28.4-41.5 | 38.8 ± 1.8 | 39.1 | 35.2-42.1 | ns |
| Albumin/globulin ratio | 1.2 ± 0.2 | 1.2 | 0.8 - 1.5 | 1.3 ± 0.2 | 1.3 | 0.9 - 1.8 | ns |
| Globulin (g/L) | 32.2 ± 7.3 | 32.9 | 18.6-44.0 | 30.1 ± 4.4 | 30.1 | 23.0 - 37.8 | ns |
| Phosphorus (mmol/L) | 2.2 ± 0.4 | 2.2 | 1.5-2.7 | 1.2 ± 0.3 | 1.2 | 0.7 - 1.6 | 0.0001 |
| Calcium (mmol/L) | 2.8 ± 0.1 | 2.8 | 2.6 - 3.0 | 2.7 ± 0.1 | 2.7 | 2.4 - 3.0 | ns |
| Magnesium (mmol/L) | 1.4 ± 0.1^{2} | 1.4 | 1.2-1.7 | 1.2 ± 0.2 | 1.2 | 0.9 - 1.4 | 0.0001 |
| Cortisol (nmol/L) | 145.2 ± 35.6 | 154.5 | 91-206 | 221.3 ± 56.2 | 215.0 | 94.0-316.0 | < 0.0001 |

¹ CK = creatine kinase, AST = aspartate aminotransferase, GGT = gamma-glutamyl transferase, ALP = alkaline phosphotase, GLD = glutamate dehydrogenase, and SDH = sorbitol dehydrogenase.

² Not normally distributed.

³ Not significant (p > 0.05).

⁴ Value not available.

three were grossly hemolyzed and three were slightly hemolyzed.

Serum Biochemistry

Serum biochemistry results from Group 1 (Kugluktuk-shot) and Group 2 (Kugluktuk-helicopter immobilized) were compared statistically. There were no differences between these two groups with respect to serum chloride, potassium, Na/K ratio, calcium, ALP, AST, total protein, globulin, albumin/globulin ratio or albumin levels (Table 3). Sodium, creatinine, phosphorus, magnesium, and creatine kinase (CK) were significantly higher in Group 1 (Kugluktuk-shot) (p = 0.004, p < 0.0001, p =0.0001, p = 0.0001, and p = 0.002 respectively). Conversely, urea, glucose, and GGT were significantly higher in Group 2 (Kugluktuk-helicopter immobilized) (p < 0.0001, p =0.028, and p = 0.001 respectively). Values for CK exceeded the upper limit (600 U/L) of the range reported for captive muskoxen by Tedesco et al. (1991b) for all muskoxen in Group 1 (Kugluktuk-shot) and 50% of the muskoxen in Group 2 (Kugluktuk-helicopter immobilized) (Table 2).

Values for AST exceeded the upper limit of the reference interval reported for captive muskoxen (108 U/L) (Tedesco et al., 1991b) in 70% of the muskoxen in Group 1 (Kugluk-tuk-shot) and 78% of the muskoxen in Group 2 (Kugluk-tuk-helicopter immobilized).

Cortisol

Serum cortisol levels in Group 2 (Kugluktuk-helicopter immobilized) were higher than in all other groups (p < 0.0001). Serum cortisol levels in Group 1 (Kugluktukshot) were not different from those in Groups 5 (Cambridge Bay-shot) or 6 (Kugluktuk-snowmobile immobilized), but cortisol levels in Group 6 (Kugluktuk-snowmobile immobilized) were significantly higher than in Group 5 (Cambridge Bay-shot) (p = 0.012) (Fig. 1).

DISCUSSION

This study provides the first report on the serum biochemistry and serum cortisol of free-ranging, adult, female



FIG. 1. Serum cortisol from muskoxen (*Ovibos moschatus*) separated into six groups depending on the location, capture method or hunted, and year of serum collection. Bars show first and third quartiles with medians (black lines), while whiskers represent 2.5th and 97.5th percentiles. Serum cortisol values were compared statistically among Groups 1 (Kugluktuk-shot, n =10), 2 (Kugluktuk-helicopter immobilized, n = 18), 5 (Cambridge Bay-shot, n = 17), and 6 (Kugluktuk-snowmobile immobilized, n = 33). See text for group details and statistical analysis. Serum cortisol values from Groups 3 (Norman Wells-helicopter immobilized) and 4 (tame) are presented for descriptive purposes only (with *).

muskoxen following immobilization or hunting. Over half of the serum samples from Group 1 (Kugluktuk-shot) and Group 2 (Kugluktuk-helicopter immobilized) were considered to be slightly or grossly hemolyzed, which occurs when hemoglobin and other cell contents are released into plasma from damaged erythrocytes (Lippi et al., 2006). While no muskoxen-specific data regarding the effect of hemolysis on serum biochemical parameters have been reported, several studies report effects on serum biochemistry of humans and domestic animals (Jacobs et al., 1992; Yucel and Dalva, 1992; Kroll and Elin, 1994; Lippi et al., 2006). In human serum, the addition of lysed blood cells causes a dose-dependent trend toward overestimation of AST, creatinine, CK, magnesium, phosphorus, potassium, and urea and underestimation of albumin, ALP, chloride, GGT, glucose, and sodium (Lippi et al., 2006). In contrast, a study by Yucel and Dalva (1992) demonstrated an increase in ALP and potassium in hemolyzed human serum samples, but did not detect an effect on AST, glucose, albumin, creatinine, magnesium, or urea. Hemolysis had no effect on any of these parameters in bovine blood (Jacobs et al., 1992). Many biochemical tests are unaffected by hemolysis, or the effects are species-specific (Wesson et al., 1979; Ehsani et al., 2008). Since hemolysis is usually preventable, it would be prudent for individuals who attempt to collect blood from free-ranging wildlife to be aware of the causes of hemolysis, ways to prevent hemolysis, and effects of hemolysis on interpretation of serum biochemical results.

Serum Biochemistry

Several differences were found in serum biochemistry values between muskoxen shot by hunters (Group 1: Kugluktuk-shot) and muskoxen captured by chemical immobilization from a helicopter (Group 2: Kugluktuk-helicopter immobilized). Unless otherwise specified, the following discussion refers to these two groups of animals. The higher serum urea concentrations of immobilized muskoxen may reflect dietary protein intake, which is highest in the summer and fall and lowest in the winter months. Muskoxen in Group 2 were immobilized in September, whereas muskoxen in Group 1 were hunted in November. The serum urea concentrations presented here are consistent with other reports of seasonal levels in muskoxen (Tedesco et al., 1991a, b). While urea concentration in serum can be an indicator of renal function, serum urea concentrations also rise and fall with nutritional status (Tedesco et al., 1991a; Morton et al., 1995; López-Olvera et al., 2006) and are reported to change seasonally in several species of wild ruminants (Bahnak et al., 1979; Tryland, 2006), including muskoxen (Tedesco et al., 1991a). Serum creatinine levels were higher in the hunted muskoxen, but the range reported for creatinine in captive muskoxen (Tedesco et al., 1991b) encompasses the ranges of both hunted and immobilized animals. Serum creatinine concentrations have been reported to change seasonally in white-tailed deer (Odocoileus virginianus) (Sakkinen et al., 2001). Elevated creatinine concentrations in captive reindeer fed lichen were attributed to low protein intake, which results in a lower glomerular filtration rate and reduced excretion of creatinine by the kidney (Sakkinen et al., 2001). In free-ranging reindeer, creatinine levels were highest in the winter, likely because of the low-protein feed available (Sakkinen et al., 2001). A similar pattern has been noted in wild muskoxen (Tedesco et al., 1991a). In this study, samples from the immobilized muskoxen and the hunted muskoxen were collected after fall plant senescence. which likely resulted in lower protein levels in the diet. Serum creatinine (Cattet et al., 2003) and urea (Montane et al., 2002) concentrations have been used as indices of physical exertion. In the present study, we were unable to determine whether the observed difference in creatinine levels between the immobilized muskoxen and hunted muskoxen was related to differences in forage quality, exertion before sampling, or effects of immobilization drugs.

The hunted muskoxen demonstrated higher serum concentrations of the muscle enzymes CK and AST compared to the immobilized muskoxen, suggesting that increased physical effort was a factor for the hunted animals. Muscle enzyme levels in serum rise as a result of increased muscle cell permeability and other damage caused by intense muscle activity (Williams and Thorne, 1996; Montane et al., 2002; Cattet et al., 2003), and elevated serum concentrations may occur with capture, restraint, and handling of wildlife (Montane et al., 2002; Cattet et al., 2003, 2008; Paterson, 2007). The levels seen in the hunted muskoxen were

| Parameter (units) ¹ | Mean ± SD | Median | Range |
|--------------------------------|---------------------|--------|-------------|
| Sodium (mmol/L) | 140.0 ± 1.6 | 141.0 | 138.0-143.0 |
| Potassium (mmol/L) | 5.7 ± 1.0 | 5.9 | 4.5-6.9 |
| Na/K ratio | 25.4 ± 4.7 | 24.0 | 20.0-32.0 |
| Chloride (mmol/L) | 98.1 ± 3.5 | 97.5 | 93.0-105.0 |
| Bicarbonate (mmol/L) | 22.3 ± 4.5 | 23.0 | 14.0-28.0 |
| Anion Gap (mmol/L) | 26.4 ± 6.7 | 23.5 | 19.0-39.0 |
| Urea (mmol/L) | 2.1 ± 1.2^2 | 1.5 | 1.3-4.8 |
| Creatinine (mmol/L) | 288.0 ± 49.0 | 284.0 | 210.0-360.0 |
| Glucose (mmol/L) | 6.8 ± 1.3 | 6.5 | 5.2-9.2 |
| CK (U/L) | 243.9 ± 76.73^2 | 223.5 | 177.0-416.0 |
| AST (U/L) | 67.0 ± 13.5 | 66.0 | 50.0-89.0 |
| GGT (U/L) | 75.0 ± 10.1 | 72.0 | 64.0-93.0 |
| ALP (U/L) | NA^3 | NA | NA |
| GLD (U/L) | 6.4 ± 4.1 | 5.5 | 2.0-15.0 |
| SDH (U/L) | 2.1 ± 2.6^2 | 1.0 | 0.0-8.0 |
| Total bilirubin (umol/L) | 5.1 ± 1.0 | 5.5 | 4.0-6.0 |
| Indirect bilirubin (umol/L) | 4.0 ± 0.7 | 4.3 | 3.1-4.7 |
| Direct bilirubin (umol/L) | 1.1 ± 0.3 | 1.1 | 0.8-1.5 |
| Cholesterol (units) | NA | NA | NA |
| Total protein (g/L) | 58.9 ± 2.7 | 59.5 | 55.0-62.0 |
| Albumin (g/L) | 38.8 ± 1.75 | 38.5 | 36.0-42.0 |
| Albumin/globulin ratio | 2.0 ± 0.3 | 1.9 | 1.58-2.47 |
| Globulin (g/L) | 20.1 ± 2.5 | 20.0 | 17.0-24.0 |
| Phosphorus (mmol/L) | 1.3 ± 0.4 | 1.4 | 0.8-1.9 |
| Calcium (mmol/L) | 2.4 ± 0.1 | 2.5 | 2.3-2.6 |
| Magnesium (mmol/L) | 1.1 ± 0.1 | 1.0 | 0.9-1.2 |
| Cortisol (nmol/L) | 115 ± 55.2 | 129.0 | 31.0-201.0 |

TABLE 4. Serum biochemistry parameters for free-ranging adult female muskoxen (O. moschatus) from Group 3, which were chemically immobilized from a helicopter near Norman Wells, Northwest Territories, between 2007 and 2009 (n = 8).

¹ CK = creatine kinase, AST = aspartate aminotransferase, GGT = gamma-glutamyl transferase, ALP = alkaline phosphotase, GLD = glutamate dehydrogenase, and SDH = sorbitol dehydrogenase.

² Not normally distributed.

³ Value not available.

higher than those in the immobilized animals, and the values from the immobilized muskoxen were comparable to those reported for captive muskoxen (Tedesco et al., 1991b). While the hunted muskoxen were not restrained, they were chased for some time and distance prior to being shot. This physical exertion may have played a role in the elevated CK and AST levels. Remote injection of drugs during chemical immobilization may cause significant muscle injury (Cattet et al., 2003); however, free-ranging grizzly bears (Ursus arctos) captured by physical means (leg-hold snares) had higher serum CK levels than bears remotely immobilized from a helicopter (Cattet et al., 2003). The serum CK ranges reported here for muskoxen are wide and the standard deviations large, which is consistent with other reports of captured wild ungulates (Marco et al., 1997; Marco and Lavin, 1999). Very high levels of CK and AST may be associated with exertional myopathy, a condition that occurs in wild mammals and birds that are severely stressed by capture, handling, or transport (Vassart et al., 1992; Montane et al., 2002), which results in degeneration and necrosis of skeletal muscle cells (Vassart et al., 1992; Williams and Thorne, 1996). Exertional myopathy can result in acute metabolic acidodsis and death in ungulates, or it may manifest as a delayed peracute syndrome, causing death of the affected animal 24 hours or more after the initial insult (Montane et al., 2002). In this study, a CK value of 8752 U/L was

documented in one muskox immobilized from a helicopter. This animal also had the highest levels of serum AST, indicating significant muscle injury either immediately before or during chemical immobilization. However, like all immobilized muskoxen included in this study, this animal was observed during and immediately after recovery from anesthesia, and no abnormalities were noted in this muskox during the immediate post-recovery phase.

The serum sodium levels reported for the hunted muskoxen and the immobilized muskoxen in this study are similar to those reported for captive muskoxen (Tedesco et al., 1991b), although levels were higher in the hunted muskoxen than in the immobilized animals. Serum sodium concentrations were higher in red deer (Cervus elaphus) captured by physical means than in those immobilized chemically (Marco and Lavin, 1999), and comparable results have been obtained in grizzly bears (Cattet et al., 2003). Serum phosphorus and magnesium were both higher in the hunted muskoxen. Elevation in serum phosphorus and magnesium are reported to occur in wild reindeer (Rangifer tarandus tarandus) during autumn, and this rise is probably related to nutrition (Nieminen, 1980); however, phosphorus and magnesium in both groups are comparable to those reported in captive muskoxen (Tedesco et al., 1991b).

In this study, the immobilized muskoxen had higher serum glucose than the hunted animals, although the difference is likely not physiologically important. Glucose levels have been reported to be elevated in ruminants captured by physical means (Spraker, 1993) and in animals immobilized with alpha-2 anaesthetics (Marais et al., 1991; Howard et al., 2004). In the former, there is a hyperglycaemic effect of catecholamines and glucocorticoids released during the stressful event of a capture, while in the latter, the drug inhibits the release of insulin and increases the output of glucose from the liver (Jalanka, 1988). Since alpha-2 antagonist drugs were not employed in this case, the hyperglycemic effect of capture may have resulted in the elevated glucose levels in immobilized animals. Because of the prolonged contact of erythrocytes with serum in the muskoxen samples (2 to 12 hours between blood collection and centrifugation), glucose concentrations presented here may be lower than concentrations in blood samples centrifuged immediately after collection. Glucose gradually decreases in serum that remains in contact with blood cells because of continued glycolysis in the cells (Stockham and Scott, 2008). In cattle, serum glucose levels begin to decline in blood samples that remain unseparated after two hours at 23°C, and lower storage temperatures (i.e., 4°C) may minimize the decrease in serum glucose (Ehsani et al., 2008).

Gamma glutamyl transferase (GGT) was higher in the immobilized muskoxen, though in general muskoxen had GGT levels that were markedly higher than those of domestic cattle (Prairie Diagnostic Services, unpubl. data). While elevated GGT levels have been reported with strenuous physical activity in other captured wild animals such as grizzly bears (Cattet et al., 2003), the GGT levels in both groups of muskoxen presented here were similar to those in captive muskoxen (Tedesco et al., 1991b) and likely represent normal values in this species.

Cortisol

Serum cortisol was compared among six groups of muskoxen: 10 free-ranging muskoxen shot from snowmobiles near Kugluktuk, Nunavut; 18 free-ranging muskoxen chemically immobilized from a helicopter near Kugluktuk, Nunavut; 8 free-ranging muskoxen chemically immobilized from a helicopter near Norman Wells, Northwest Territories; 5 tame muskoxen maintained on pasture; 17 free-ranging muskoxen shot from snowmobiles near Cambridge Bay, Nunavut; and 33 free-ranging muskoxen chemically immobilized from a snowmobile. Serum cortisol was highest in Group 2 (Kugluktuk - helicopter immobilized), which likely indicates a higher stress response in this group. Plasma or serum cortisol concentration has been used as an indicator of stress in many free-ranging and captive wildlife species (Wesson et al., 1979; DelGiudice et al., 1990; Chapple et al., 1991; Morton et al., 1995; Marco et al., 1997; Read et al., 2000). Blood cortisol levels from shot animals have been used as baseline levels in some studies (Cheney and Hattingh, 1988); however, in this study, hunted

muskoxen in one group had cortisol values similar to those of some groups of chemically immobilized animals. This pattern suggests that tame captive animals may provide a better blood cortisol baseline for wild species than hunted animals.

Although differences in the processing and analysis of serum from some of the muskox groups in this study, as well as the small sample sizes in some groups, present challenges for drawing definite conclusions about the effects of immobilization on serum biochemistry in muskoxen, this study provides evidence of some differences in the serum biochemistry and serum cortisol between hunted and immobilized muskoxen. Despite the limitations, these data provide a valuable description of clinical biochemistry in wild free-ranging muskoxen, particularly because they represent muskoxen that were captured using different methods and from different locations in the Canadian North. These data will be beneficial when assessing diseased or healthy individuals or populations. Developing serum cortisol and biochemical baseline reference ranges for free-ranging muskoxen would require a large number of samples collected from healthy animals that represent the entire population in terms of age, sex, habitat, and season (López-Olvera et al., 2006). Samples for establishing such values may be difficult or impossible to obtain in free-ranging wildlife, as the stress of the sampling, physical exertion, chemical immobilization, and capture may all affect the outcomes (Tryland, 2006). Therefore, the values reported here contribute to the body of available physiological data for muskoxen and will support further research into the effects of immobilization and other health assessments on this species.

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