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# Serum Chemistry Values of Free-ranging, Lactating Northern Fur Seals (*Callorhinus ursinus*)

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ABSTRACT: Reference range clinical serum chemistry values were established for free-ranging lactating northern fur seals (*Callorhinus ursinus*). Fur seals sampled for this study were part of a healthy and growing population in the Kuril Islands of far-east Russia. Blood was collected from 45 females between June and August 2005 to 2007. Fresh serum was assayed for 16 components. Packed-cell volume was determined from fresh whole blood. Results are made available for future comparisons with the declining population of northern fur seals on the Pribilof Islands and are compared with published values for other otariid species.

*Key words:* *Callorhinus ursinus*, female, free-ranging, lactating, northern fur seal, serum chemistry.

Northern fur seal (*Callorhinus ursinus*) populations on the Pribilof Islands, Alaska, are declining at an alarming rate. The entire stock of Pribilof Islands fur seals has been listed as *depleted* under the Marine Mammal Protection Act (Loughlin et al., 1994), and pup production has been dropping approximately 6% per year since 1998 (Towell et al., 2006). Factors contributing to the decline are not clear, but exposure to environmental organochlorine contaminants and its effects on reproductive rates and pup survival have been implicated as a potential cause (Beckman et al., 1999). Exposure to chemical pollutants can suppress immune and endocrine functions, leading to increased susceptibility to injury and disease, metabolic disorders, and lowered reproductive rates (O'Hara and O'Shea, 2001). Tracking changes in serum chemistry profiles and comparing them to reference range clinical values is a valuable tool for detecting the presence of disease or nutritional stress in individual animals and can provide early indication of potential health problems in at-risk populations. Published serum chemistry values are currently available for northern fur seal pups and bulls (Hubbard, 1968; Hunter and Madin, 1976) but have not been established for lactating females. Here, we report the results of 3 yr of blood collection and analyses for free-ranging, lactating, female northern fur seals from a growing population considered to be healthy, located along the far-east coast of Russia.

Northern fur seals were eliminated from the Kuril Islands by uncontrolled harvests in the late 19th century and rookeries were not reestablished until 1955 (Tikhenko, 1914; Klumov, 1957). Currently the population exceeds more than 100,000 seals and has been growing with an annual increase in pup production of 4% from 1988 to 2006 (Burkanov et al., 2007). The primary objective of this report is to provide a panel of reference range serum chemistry values for healthy, lactating northern fur seals, which may be valuable in the determination of causative factors for the population decline of northern fur seals on the Pribilof Islands.

Serum samples were collected from 45 lactating, female northern fur seals between June and August 2005 to 2007. Seals in this study were captured on Lovushki Island (48°33′14.4″N, 153°51′25.2″E), a rookery that is part of the Kuril Island chain located along the far-east coast of Russia. The work

was conducted under permits from the Russian regional permitting agency SakhalinVetSanNadzor and was approved by the Alaska Sealife Center Institutional Animal Care and Use Committee. Females were selected based on the presence of a suckling pup, then captured on the rookery using hoop-nets. Captured seals were transferred to a research ship, where they were weighed and held under minimal restraint until isoflurane anesthesia could be administered. Once anesthetized, seals underwent a physical assessment to identify visible injuries or abnormalities (e.g., broken bones, lesions, discharge). Venipuncture was performed on an interdigital vein located on the dorsal surface of the hind or fore flipper using a 1.9-cm, 19-gauge, winged infusion set attached to a 12-ml slip-tip disposable syringe. Blood for serum chemistries was transferred into 6-ml evacuated serum separator tubes, whereas blood for packed-cell volume (PCV) measurement was transferred into 4-ml, evacuated tubes containing ethylenediaminetetraacetic acid (EDTA) and gently rocked. Packed-cell volume was determined immediately after collection using a plain capillary tube centrifuged at 4,400 × G for 5 min and a capillary-tube card reader. Serum separator tubes were allowed to clot for 20 min at room temperature before centrifugation at 1,315 × G for 15 min. Serum aliquots were collected into 1.2-ml cryogenic vials. Fresh serum samples were assayed using a VetScan® Classic analyzer with comprehensive diagnostic and thyroid profile rotors (Abaxis North America, Union City, California, USA) to determine the following clinical serum chemistry parameters: albumin, alkaline phosphatase, alanine amino transferase, amylase, total bilirubin, blood urea nitrogen (BUN), calcium, phosphorus, creatinine, glucose, sodium, potassium, total serum proteins, globulins, thyroxin ($T_4$), and cholesterol. The VetScan Classic is a spectrophotometry-based blood-chemistry analyzer widely used among researchers for establishing and monitoring blood chemistry values in a variety of mammals and exotic species in both a field and laboratory setting (Mayer et al., 2005; Mellish et al., 2006, 2007; Johnston et al., 2007). The VetScan Classic has built-in quality-control mechanisms for assuring that the reagents used for analyses fall within an expected range and have not been compromised by temperature or humidity. If physical interference, such as hemolysis, is detected in the sample at a level that will adversely effect results for the analyte being measured, the values will not be printed.

Of the 45 seals included in this study, 14 individuals (31%) were sampled twice within their respective breeding season. When multiple serum chemistry and PCV values were available for a single animal within the same breeding season, they were averaged to produce a single value for that individual. The mean and standard deviations reported here are for all individual mean values across 3 yr, whereas the range includes the absolute minimum and maximum values observed for all samples from all animals in all years (Table 1).

Results were obtained from seals considered to be healthy and in good body condition, with no visual signs of disease or injury. Seals displaying injuries, abnormalities, or a PCV outside of the reference range listed for captive California sea lions were excluded from the data set. White blood cell (WBC) counts were available from blood smears for 21 seals (47%) in the study. The WBC counts for our seals were within range of values reported for captive California sea lions and lactating Australian sea lions (Needham et al., 1980; Bossart et al., 2001). Because cell counts were not available for all seals, globulin, PCV, and total protein values from seals with WBC counts were compared with seals without WBC counts using a two-sample, Student's *t*-test ($\alpha=0.05$). These parameters were chosen for comparison because they are often used as indicators of an immune response and are not easily altered by an acute stress response or influenced by the nutrient composition of

TABLE 1. Mean, standard deviation, and range of serum chemistry values for lactating northern fur seals (*Callorhinus ursinus*) and published values for other otariid species.

| Serum parameter[a] | Free-ranging, lactating northern fur seals (*Callorhinus ursinus*, $n=45$)[b] | | | Free-ranging, bull northern fur seals (*Callorhinus ursinus*; $n=20$)[c] | Free-ranging, female Australian sea lions (*Neophoca cinerea*; $n=19$)[d] | Captive California sea lions (*Zalophus californianus*; $n=23$)[e] |
|---|---|---|---|---|---|---|
| | Mean (SD) | Min | Max | | | |
| Albumin (g/dl) | 4.65 (0.42) | 3.5 | 5.3 | ND | ND | 2.7–3.6 |
| Alkaline phosphatase (U/l) | 61.3 (23.00) | 36.0 | 170.0 | 31–89 | 62 (23) | 34–175 |
| Alanine amino transferase (U/l) | 45.1 (17.11) | 22.0 | 94.0 | ND | ND | 19–71 |
| Amylase (U/l) | 510.2 (146.59) | 304.0 | 895.0 | ND | ND | ND |
| Total bilirubin (mg/dl) | 0.36 (0.05) | 0.3 | 0.5 | 0.3–9.0 | 0.2 (0.05) | 0.1–0.4 |
| BUN (mg/dl) | 25.74 (7.12) | 14.0 | 54.0 | 29.5–49.9 | ND | 14–38 |
| Calcium (mg/dl) | 9.64 (0.40) | 8.3 | 10.7 | ND | 8.92 (1.58) | 8.8–11.5 |
| Phosphorus (mg/dl) | 6.20 (1.43) | 2.8 | 10.1 | 4.0–7.2 | 6.42 (1.28) | 1.8–7.8 |
| Creatinine (mg/dl) | 0.85 (0.23) | 0.3 | 1.4 | 1.0–1.7 | 1.13 (.34) | 1.1–2.6 |
| Glucose (mg/dl) | 168.4 (24.14) | 123.0 | 249.0 | 12–187 | 73.8 (19.26) | 71–203 |
| Sodium (mmol/l) | 149.3 (2.76) | 139.0 | 156.0 | ND | 145 (17.5) | 149–156 |
| Potassium (mmol/l) | 4.43 (0.34) | 3.6 | 5.2 | ND | 4.5 (0.75) | 3.7–5.0 |
| Total protein (g/dl) | 7.33 (0.55) | 6.1 | 9.2 | 6.4–8.2 | 6.9 (1.3) | 6.1–8.5 |
| Globulin (g/dl) | 2.68 (0.50) | 1.5 | 4.9 | 3.1–4.8 | ND | 3.1–5.6 |
| PCV | 45.1 (4.00) | 38 | 56.5 | ND | ND | 38–59 |
| Cholesterol (mg/dl) | 286.2 (19.0) | 245.0 | 309.0 | 188–509 | ND | 165–507 |
| $T_4$ (μg/dl) | 2.85 (0.81) | 1.9 | 4.5 | ND | ND | ND |

[a] BUN = blood urea nitrogen; PCV = packed cell volume; $T_4$ = thyroxin; ND = no data.

[b] Mean = the pooled means for all animals across all years; min = the minimum of minimum values across all animals and years; max = the maximum of maximum values across all animals and years; SD = standard deviation, Mean and range values for cholesterol and $T_4$ concentrations represent values obtained from seven individuals between 2005 and 2006.

[c] Hunter and Madin, 1976.

[d] Cargill et al., 1979; chemistry values obtained from fresh plasma.

[e] Bossart et al., 2001.

prey items ingested and time between feeding and sample collection. There were no significant differences between the two groups. Therefore, we are confident that these groups are similar and, together, represent a healthy subgroup of a geographic population of free-ranging northern fur seals in a specific life stage (lactation and reproduction). These values are provided as reference data to allow future comparisons of blood chemistry data from other adult, female fur seals, which will assist in the health assessment and management of both free-ranging and captive fur seals.

The mean and range for serum components of lactating northern fur seals were similar to published values for other otariids (Table 1), except for albumin, globulin, and glucose. In general, blood values listed as within reference range for a species are considered to be guideline values. The only sure method for determining a value to be out of reference range or for diagnosing disease is by establishing baseline values for an individual and tracking changes in those values over time (Bossart et al., 2001). Differences between the values reported here and published values for other otariids may be due to several factors, including the use of different automated analyzers or laboratory techniques; variation in sex, age, and life stage; differences in diet composition and fed or fasted state; and the physiologic response to restraint and handling. For the seals in our study, we could not determine how much time had elapsed between a seal's last meal and blood collection, but some seals were

undoubtedly sampled shortly after returning from a foraging trip and were in a fed state whereas others had been hauled out on land, fasting and nursing a pup for several days. Captive animals are almost always fasted before anesthesia and blood collection, so it is likely that differences in the time between feeding and sample collection caused some of the variability in serum chemistry values we observed.

The range in albumin concentration for lactating northern fur seals was high (3.5–5.3 g/dl) when compared with captive adult California sea lions (*Zalophus californianus*; 2.7–3.6 g/dl). It is possible that dehydration due to loss of body water to milk production or an immune response to inflammation could have contributed to the elevated albumin values we found in lactating fur seals. However, PCV and other serum chemistry parameters, such as globulins and total protein concentrations, which should also indicate dehydration or an inflammatory response, were within the range of values reported for captive California sea lions. Because albumin concentrations are directly related to protein intake, the most likely explanation for the higher albumin levels presented here is differences in diet composition and time between feeding and sample collection of free-ranging versus captive animals (Bossart et al., 2001; Mellish et al., 2006). Globulin concentrations reported in this study were slightly lower (1.5–4.9 g/dl) when compared with captive California sea lions (3.1–5.6 g/dl); the reason for this is unclear. One possible explanation for the lower globulin concentrations reported here would be the transfer of immunoglobulins from the female's serum into the milk to provide passive immunity to the pup (Bossart et al., 2001; Marquez et al., 2003).

Blood-glucose concentrations presented here for lactating fur seals were elevated (123–249 mg/dl) when compared with the other otariids (Table 1). The cause for this is unknown. Stress associated with handling and restraint during venipuncture has been shown to cause increased blood-glucose concentrations from the release of endogenous glucocorticoids (Bossart et al., 2001). The degree to which the effects of stress contribute to variation in serum chemistry values will differ between a captive animal that is accustomed to restraint and a naïve, free-ranging seal. Because the seals sampled for our study are wild, restraint was necessary to obtain a blood sample. Therefore, it is possible that stress may have contributed to some of the variation seen in our results. In an effort to minimize the effects of stress associated with restraint and handling, all of our seals were anesthetized before venipuncture, so total restraint time, while conscious, was probably shorter than in other studies with free-ranging otariids. The mean amylase value we report here for lactating northern fur seals (510.2 U/l) was high compared with a mean of 145 U/l reported for Steller sea lion (*Eumetopias jubatus*) juveniles and pups (132 U/l; Mellish et al., 2006); the cause of this is unknown. Although amylase is often used to diagnose diseases commonly seen in companion animals, few publications report values for amylase concentrations in pinnipeds. Amylase concentrations are often used, in combination with lipase, as markers for acute pancreatitis and chronic renal insufficiency (Bossart et al., 2001). The ability to assess amylase concentrations is practical and may be important for performing health assessments in pinnipeds, such as fur seals, especially with captive animals, where repeated blood collection is possible and changes in amylase concentrations over time can be monitored. Mean and range values for cholesterol and $T_4$ concentrations presented in this study represent values obtained from seven individuals between 2005 and 2006. Means for both cholesterol (286.2 mg/dl) and $T_4$ (2.8 μg/dl) concentrations are similar to values previously reported for northern fur seals (319 mg/dl and 2.8 μg/dl) of various ages and both sexes (Hunter and Madin, 1976; St. Aubin, 2001).

In summary, the serum chemistry values not specifically mentioned above were all similar to values reported for other otariids. This is the first study, to our knowledge, to present serum chemistry values on free-ranging, lactating northern fur seals. These values should prove useful in providing normal serum chemistries as reference for assessing the health of both wild and captive fur seals as well as to provide valuable information on potential causes contributing to the decline of populations at risk.

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