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BIOCHEMICAL AND HEMATOLOGIC REFERENCE VALUES FOR FREE-RANGING, CHEMICALLY IMMOBILIZED WILD NORWEGIAN REINDEER (*RANGIFER TARANDUS TARANDUS*) DURING EARLY WINTER

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ABSTRACT: Hematologic and serum biochemistry values were evaluated in free-ranging, wild Norwegian reindeer (*Rangifer tarandus tarandus*) as part of a reintroduction program in southwestern Norway in November 1995 and 1996. Animals were immobilized with medetomidine-ketamine by dart from a helicopter. Blood was drawn for serum chemistry from 31 adults (nine males and 22 females) and for hematology from 29 adults (eight males and 21 females). Significant differences ($P<0.05$) were found between male and female results for alkaline phosphatase, selenium, and zinc. Although there was a significant difference between male and female gamma-globulin values and the total albumin:globulin ratio, the overall values are much lower than those reported for other *Rangifer* species. Sexual differences should be interpreted with caution due to the low number of males compared to females. References ranges are presented combining male and female results for hematology and serum chemistry and separately for males and females for serum electrophoresis. No correlation was found between induction time and aspartate transaminase, creatine kinase, glucose, cortisol, or total protein. Blood values were generally similar to those published for semidomestic reindeer (*Rangifer tarandus tarandus*) and free-ranging caribou (*Rangifer tarandus caribou*), but the effect of capture drugs, stress, season, and sample size should be considered with interpretation. This paper provides the first report of baseline hematologic and serum biochemistry reference ranges for free-ranging, wild Norwegian reindeer during early winter.

Key words: Blood, hematology, *Rangifer tarandus*, reindeer, serum chemistry, serum electrophoresis.

INTRODUCTION

In Europe, free-ranging, wild reindeer (*Rangifer tarandus tarandus*) exist only in Southern Norway. However, reindeer avoidance of human infrastructure, such as power lines, roads, and mountain cabins, has created separated subpopulations that face increased loss of habitat and isolation as this infrastructure continues to expand (Nellemann et al., 2001, 2003). In recent years, risk of disease transmission in Fennoscandic reindeer has also increased due in part to increased interaction with livestock, increased animal movements across borders, and changing climate (Tryland, 2012). These population threats have enhanced the need for research and management of the population. We previously described an effective chemical immobilization technique in Norwegian wild reindeer (Arnemo et al., 2011); here, we present normal blood values useful for health assessment in free-ranging, wild reindeer.

Obtaining blood samples is noninvasive and easily done in the field if animals are captured, but the results from analysis are difficult to interpret without well-established reference ranges. Although reference ranges exist for some wild species (Johnson et al., 2010), the cost and logistics of sampling enough individuals to validate a

reference range is often prohibitive in wildlife. Comparisons made to domestic individuals of the same species or a similar species do not account for species variation or differences between captive and wild individuals. Particularly in wild individuals, variables influenced by capture techniques and stress can cause significant changes in blood parameters (Arnemo et al., 1994; Marco and Lavín, 1999). Although reference values have been reported for captive, semidomesticated reindeer (*Rangifer tarandus tarandus*; Nieminen, 1980; Nieminen and Timisjärvi, 1981, 1983; Catley et al., 1990), this is the first report of hematologic and biochemical reference values for free-ranging, wild Norwegian reindeer in early winter.

MATERIALS AND METHODS

Study site and animals

As part of a reintroduction program, 32 adult, wild Norwegian reindeer (23 females and nine males) were captured and translocated from Nordfjella (61°57′N, 07°54′E) to Lærdal-Årdal (61°06′N, 07°34′E) in Sogn and Fjordane County, southwestern Norway, in November 1995 and 1996. Animals were darted with a combination of medetomidine and ketamine from a helicopter as described (Arnemo et al., 2011). Animals chosen for capture were determined to be healthy adults (≥1.5 yr). Experienced reindeer hunters estimated the body mass of each animal. Ethical approval for this research was given by the Norwegian Animal Research Authority (Oslo, Norway) and the Norwegian Directorate for Nature Management (Trondheim, Norway).

Specimens and laboratory analyses

Blood samples for hematology and serum chemistry were collected from the jugular vein within 30 min of immobilization. Methods for sample collection and laboratory analysis have been described (Rostal et al., 2012). Parameters recorded for the complete blood count (CBC) included white blood cell count (WBC), red blood cell count, hemoglobin, hematocrit (HCT), mean corpuscular volume, mean corpuscular hemoglobin (MCH), MCH concentration, red cell distribution width, hemoglobin cell distribution width, platelet count, and mean platelet volume. A white cell count differential (absolute numbers and percent of total) was included for neutrophils, lymphocytes, monocytes, eosinophils, basophils, and large unstained cells. Serum chemistry parameters included aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), lactate dehydrogenase, gamma-glutamyl transpeptidase, glutamate dehydrogenase, amylase, lipase, total protein, urea, creatinine, uric acid, total bilirubin, cholesterol, triglycerides, free fatty acids, β-hydroxybutyrate, glucose, phosphorus, calcium, magnesium, sodium, potassium, chloride, iron, copper, zinc, selenium, and cortisol. Serum protein electrophoresis included albumin, alpha-globulins, beta-globulins, gamma-globulins, and the albumin-globulin (A:G) ratio.

Data analysis

Statistical analyses were performed using JMP® statistical software (SAS Institute, Carey, North Carolina, USA). A P value of <0.05 was considered significant. Normal distributions were tested for males and females using a Shapiro-Wilk test. If both sexes were normally distributed, an unpaired, two-tailed Student's t-test was used for comparison. If both sexes were not normally distributed, a Kruskal-Wallis test was used.

Reference ranges for CBC and serum chemistry were determined as described for nonparametric data (Lassen, 2006a). Briefly, outliers were identified as values greater than one third of the range of the entire data set from the next closest data point. Reference ranges are reported with outliers removed. Mean, median, and standard error are reported for the entire sample set. Data are presented as mean (median)±SE unless otherwise stated.

For induction time analysis, only animals in which serum chemistry was successfully collected were used. Induction time was defined as the time between darting and the animal becoming recumbent. Animals that received more than one dart were excluded ($n=3$), leaving 28 animals for analysis. Spearman correlation was used to determine the correlation between induction time and AST, ALT, CK, total protein, glucose, and cortisol levels.

RESULTS

Thirty adult (>1.5 yr), wild Norwegian reindeer (23 females and nine males) were sampled. Animal weights were 50–90 kg (Arnemo et al., 2011). Not all tests could be performed on each animal. Hematology results were obtained for CBC from 29 animals (21 females and eight males). No

TABLE 1. Hematology values for Norwegian free-ranging, wild reindeer (*Rangifer tarandus tarandus*) captured in southwestern Norway in November 1995 and 1996. Ranges are reported with outliers removed.

Hematologic parameters[a] (SI units)	Range	*n*	Mean±SE	Median	*n*
RBC ($\times 10^9$/L)	9.24–11.96	28	10.70±0.17	10.60	29
HGB (g/L)	145–211	29	173±2.58	173	29
HCT (L/L)	0.39–0.57	29	0.47±0.01	0.48	29
MCV (fL)	40.3–48.0	29	44.4±0.3	44.3	29
MCH (pg)	15.0–17.5	29	16.3±0.1	16.3	29
MCHC (g/L)	351–384	29	366±2	366	29
RDW (%)	13.0–16.4	29	14.4±0.2	14.2	29
HDW (g/L)	19.9–40.3	29	24.9±0.8	23.8	29
PLT ($\times 10^9$/L)	74–603	29	253±24	225	29
MPV (fL)	3.4–6.9	29	5.2±0.2	5.5	29
WBC ($\times 10^9$/L)	0.96–5.19	29	2.96±0.19	2.86	29
Neutrophils ($\times 10^9$/L)	0.56–4.52	26	1.91±0.18	1.75	26
Neutrophils (%)	37.5–87.2	19	62.0±2.8	62.5	19
Lymphocytes ($\times 10^9$/L)	0.37–1.69	26	0.90±0.06	0.87	26
Lymphocytes (%)	8.9–53.3	19	31.4±2.6	29.5	19
Monocytes ($\times 10^9$/L)	0.0–0.03	26	0.01±0.01	0.01	26
Monocytes (%)	0.0–1.3	19	0.4±0.1	0.4	19
Eosinophils ($\times 10^9$/L)	0.00–0.01	26	0.00±0.00	0.00	26
Eosinophils (%)	0.0–0.4	19	0.1±0.0	0.0	19
Basophils ($\times 10^9$/L)	0.01–0.13	26	0.05±0.01	0.04	26
Basophils (%)	0.5–4.7	19	1.5±0.3	1.1	19
LUC ($\times 10^9$/L)	0.03–0.33	26	0.16±0.01	0.15	26
LUC (%)	2.4–7.5	18	5.26±0.5	4.5	19

[a] RBC = red blood cell count; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = MCH concentration; RDW = red cell distribution width; HDW = hemoglobin cell distribution width; PLT = platelet count; MPV = mean platelet volume; WBC = white blood cell count, LUC=large unstained cells.

significant differences were found between male and female values; therefore, results were combined for male and female hematologic values (Table 1). Serum biochemistry results were obtained from 31 animals (22 females and nine males) and serum electrophoresis from 30 (21 females and nine males). Significant differences were found between males and females for ALP (female 227 [195]±23 U/L, n=22; male 332 [346]±31 U/L, n=9; P=0.015), selenium (female 0.22 [0.22]±0.01 μg Se/g blood, n=11; male 0.19 [0.19]±0.01 μg Se/g blood, n=6; P=0.033), and zinc (female 10 [11]±0 μmol/L, n=22; male 12 [12]±1 μmol/L, n=8; P=0.032). Male gamma-globulin values (9.70 [9.79]±0.75, n=9; P=0.004) were significantly higher than female values (6.72 [6.88]±0.45, n=21). This difference was reflected in significantly different total A:G ratios (female 2.76 [2.66]±0.11, n=21; male 2.19 [2.13]±0.16, n=9; P=0.009). Results for combined male and female serum biochemistry values are shown in Table 2 and for separate male and female serum electrophoresis values in Table 3.

Twenty-eight animals (20 females and eight males) were used for the induction analysis. Induction time was a mean of 8 (7)±1 min; range 1–18.5 min. No significant correlation was found for AST (ρ=−0.022, P=0.911), ALT (ρ=−0.061, P=0.758), CK (ρ=0.076, P=0.701), total protein (ρ=0.150, P=0.445), glucose (ρ=−0.080, P=0.686), or cortisol (ρ=−0.092, P=0.649). Results are shown in Table 4.

DISCUSSION

Although these reference ranges have been established on fewer than the

TABLE 2. Serum biochemical values for Norwegian free-ranging, wild reindeer (*Rangifer tarandus tarandus*) captured in southwestern Norway in November 1995 and 1996. Ranges are reported with outliers removed.

Biochemical parameters[a] (SI units)	Range	*n*	Mean±SE	Median	*n*
AST (U/L)	57–211	31	104±6	96	31
ALT (U/L)	22–77	31	38±2	34	31
ALP (U/L)[b]	100–483	31	257±20	231	31
CK (U/L)	130–928	31	356±37	320	31
LD (U/L)	644–1671	31	961±41	923	31
GGT (U/L)	10–49	31	24±2	22	31
GD (U/L)	0–10	29	6±2	3	31
Amylase (U/L)	12–76	30	45±3	47	30
Lipase (U/L)	5–30	29	14±1	13	30
Total Protein (g/L)	52–71	31	62±1	61	31
Urea (mmol/L)	1.5–15.0	31	4.5±0.6	3.1	31
Creatinine (μmol/L)	148–229	31	178±3	178	31
Uric acid (μmol/L)	0–34	31	4±2	0	31
Total bilirubin (μmol/L)	1–5	30	2±0	2	30
Cholesterol (mmol/L)	1.0–2.0	31	1.5±0.0	1.5	31
Triglycerides (mmol/L)	0.1–0.5	31	0.2±0.0	0.2	31
Free fatty acids (mmol/L)	0.1–1.3	31	0.6±0.1	0.5	31
B-HBA (mmol/L)	0.4–1.5	30	0.8±0.1	0.7	31
Glucose (mmol/L)	0.9–14.8	31	6.7±0.7	6.4	31
Phosphorus (mmol/L)	0.7–2.2	30	1.4±0.1	1.3	31
Calcium (mmol/L)	2.2–2.7	31	2.4±0.0	2.4	31
Magnesium (mmol/L)	0.69–1.04	31	0.88±0.02	0.90	31
Sodium (mmol/L)	136–153	31	144±1	144	31
Potassium (mmol/L)	2.6–4.4	30	3.6±0.1	3.5	31
Chloride (mmol/L)	96–107	31	101±0	101	31
Iron (μmol/L)	16–39	30	27±1	26	30
Copper (μmol/L)	7–14	29	11±0	10	30
Zinc (μmol/L)[b]	8–14	30	11±0	11	30
Cortisol (nmol/L)	110–477	30	299±15	300	30
Selenium (μg Se/g blood)[b]	0.17–0.27	17	0.21±0.01	0.20	17

[a] AST = aspartate aminotransferase; ALT = alanine transaminase; ALP = alkaline phosphatase; CK = creatine kinase; LD = lactate dehydrogenase; GGT = gamma-glutamyl transpeptidase; GD = glutamate dehydrogenase; B-HBA = β-hydroxybutyrate.

[b] Denotes serum analytes whereby a significant difference was noted between males and females.

recommended 40 individuals (Lassen, 2006a), it is difficult to collect samples from large numbers of free-ranging wildlife. In addition, our sample period is specific to 1 mo, November, during 2 yr. Hematologic and biochemical parameters in free-ranging wildlife change seasonally due to variation in nutrient availability. This has been observed for semidomestic reindeer (Hyvärinen et al., 1975; Nieminen, 1980), Svalbard reindeer (*Rangifer tarandus platyrhynchos*; Nilssen et al., 1985), and in free-ranging white-tailed deer (*Odocoileus virginianus*; DelGiudice et al., 1992). Furthermore, Säkkinen et al. (2001) suggests that reindeer renal physiology, and consequently plasma creatinine, may have an innate seasonality regardless of nutrition and captive or free-ranging status. Potential effects of seasonality on our findings are discussed below within the context of our results.

We found no differences between males and females for most parameters. While late-term pregnancy and parturition can cause changes in blood values such as HCT and total protein (Nieminen, 1980; Soveri et al., 1999), the female reindeer in this study, if pregnant, would have been captured very early in gestation. Selenium

TABLE 3. Serum electrophoresis values for Norwegian free-ranging, wild reindeer (*Rangifer tarandus tarandus*) captured in southwestern Norway in November 1995 and 1996. Ranges are reported with outliers removed.

Serum electrophoresis	Range	*n*	Mean±SE	Median	*n*	*P* value
Albumin (g/L)						
Female	40.19–50.38	21	44.47±0.61	44.35	21	0.128
Male	39.28–46.70	9	42.99±0.70	43.01	9	
Alpha-globulin (g/L)						
Female	4.29–7.89	21	5.27±0.22	5.03	21	0.353
Male	4.20–7.24	9	5.64±0.32	5.31	9	
Beta-globulin (g/L)						
Female	3.69–6.96	21	4.64±0.19	4.48	21	0.405
Male	3.25–6.62	9	4.99±0.35	5.16	9	
Gamma-globulin (g/L)						
Female	3.97–12.21	21	6.72±0.45	6.88	21	0.004[a]
Male	7.20–14.08	9	9.70±0.75	9.79	9	
Total albumin:globulin						
Female	1.74–3.76	21	2.76±0.11	2.66	21	0.009[a]
Male	1.50–3.00	9	2.19±0.16	2.13	9	

[a] Denotes a statistically significant difference between female and male values ($P<0.05$).

and zinc levels measured in semidomestic reindeer meat have been linked to pasture quality, animal age, and herd density (Hassan et al., 2012). Although Hassan et al. (2012) did not detect a statistically significant difference between males and females, the differences noted here between males and females for selenium and zinc could reflect subtle differences in foraging strategy between sexes. Furthermore, Hassan et al. (2012) found that young animals (1.5 yr) had lower selenium values than older animals (>2 yr). In our study eight of nine males were estimated to be 1.5–2.5 yr, while 21 of 23 females were estimated to be 2.5 yr and older. Therefore, the lower selenium values we report for males may also be due to age differences. Alkaline phosphatase is an enzyme that most often originates from the liver and can be increased in certain disease processes, particularly those affecting the liver (Lassen, 2006b). Serum levels may also be increased in young growing animals due to osteoblastic activity (Lassen, 2006b). The difference in ALP noted here is small but may reflect the age difference between males and females as discussed above. Although males had a significantly higher gamma-globulin

TABLE 4. Correlation between induction time (range 1–18.5 min.) and selected blood parameters in Norwegian free-ranging, wild reindeer (*Rangifer tarandus tarandus*) captured in southwestern Norway in November 1995 and 1996.

Variable[a]	ρ	*P* value	*n*
AST (U/L)	−0.022	0.911	28
ALT (U/L)	−0.061	0.758	28
CK (U/L)	0.076	0.701	28
Total protein (g/L)	0.150	0.445	28
Glucose (mmol/L)	−0.080	0.686	28
Cortisol (nmol/L)	−0.092	0.649	28

[a] AST = aspartate aminotransferase; ALT = alanine transaminase; CK = creatine kinase.

value than females, both male and female levels are much lower than those reported for wild, female, boreal caribou (*Rangifer tarandus caribou*) by Johnson et al. (2010) and captive, female, semidomestic reindeer by Nieminen and Timisjärvi (1983). However, any statistical difference between sexes in our study should be interpreted with caution due to the low number of males compared to females.

Our results for serum biochemistry are similar to the *Rangifer* species values reported by Flach (2003), caribou by Johnson et al. (2010), and semidomestic reindeer values by Nieminen and Timisjärvi (1983) with a few exceptions. Compared to values reported by Nieminen and Timisjärvi (1983), our mean value for blood urea concentration is low, but it is higher than the values reported by Johnson et al. (2010). Diet is an important factor for blood urea values, and low blood urea concentrations have been reported in semidomestic reindeer under poor nutritional conditions (Hyvärinen et al., 1975; Säkkinen et al., 2001). Reindeer described by Nieminen and Timisjärvi (1983) were hand fed or free grazing on pasture, but reindeer described in Johnson et al. (2010) and our study were free ranging. A seasonal effect has also been noted for blood urea with lower values occurring during autumn and winter (Hyvärinen et al., 1975). Differences in diet and sampling season may also be reflected in our lower total protein levels as compared to those reported by Nieminen and Timisjärvi (1983) and Johnson et al. (2010). Decreased total protein values are correlated with decreased gamma-globulin levels in reindeer (Hyvärinen et al., 1975; Nieminen and Timisjärvi, 1983) and may explain the low gamma-globulin levels we report. Our mean for mineral concentrations (zinc, copper, and magnesium) are comparable to the slightly lower means reported by Nieminen and Timisjärvi (1983).

Our mean values for glucose, CK, AST, ALP, and cortisol are relatively higher than those reported for semidomestic reindeer (Nieminen and Timisjärvi, 1983) but comparable to those found in free-ranging boreal caribou (Johnson et al., 2010). Increased values for these parameters are known to be related to stress (Spraker, 1993), and marked increases in AST and cortisol have been reported in experimentally stressed semidomestic reindeer (Rehbinder and Edqvist, 1981). Furthermore, the use of medetomidine has been shown to cause increases in glucose and cortisol levels in captive, semidomestic reindeer (Arnemo and Ranheim, 1999; Soveri et al., 1999).

Our results for CBC values are comparable to *Rangifer* species (Flach, 2003) and captive reindeer (*Rangifer tarandus*; Catley et al., 1990), but our mean overall white blood cell as well as individual counts for monocytes and eosinophils are notably lower than values reported in semidomestic reindeer by Nieminen and Timisjärvi (1983). While low total WBCs may be due to chronic infection or stress, the decreases seen here are likely due to drug effects. Marco and Lavín (1999) found a significantly lower total white blood cell, lymphocyte, monocyte, neutrophil, and eosinophil count in red deer (*Cervus elaphus*) chemically captured with xylazine-ketamine than in red deer captured with long nets and box traps. Similar findings were shown in red deer chemically captured with medetomidine-ketamine compared with red deer restrained in a chute (Arnemo et al., 1994).

Hyperglycemia and a decreased packed cell volume (PCV) have been reported in markhors (*Capra falconeri megaceros*), snow leopards (*Panthera uncia*), and blue fox (*Alopex lagopus*) with use of medetomidine-ketamine (Jalanka and Roeken, 1990). Medetomidine causes a hyperglycemic effect by inhibiting insulin release and a decreased PCV as a result of erythrocyte pooling in the spleen (Jalanka and Roeken, 1990; Jalanka, 1993). Arnemo and Ranheim (1999) noted a hyperglycemic response with an increase in serum glucose levels to nearly 16 mmol/L 1 hr

after injection of medetomidine and just before atipamezole was administered in captive reindeer. Our highest reported glucose value (14.8 mmol/L) is comparable to this. However, glucose values can be influenced by many factors, such as nutrition and stress, which is evident in the wide range (0.9–14.8 mmol/L) of values we obtained. Arnemo et al. (1994) noted a significantly lower PCV in red deer chemically captured with medetomidine-ketamine compared with those physically captured, but this effect was not appreciated in our study. The PCV results reported here are comparable to those reported for *Rangifer* species (Nieminen and Timisjärvi, 1981; Catley et al., 1990; Flach, 2003).

Our mean potassium level is slightly lower than levels described by Johnson et al. (2010) and Nieminen and Timisjärvi (1983), and our phosphorus level is slightly lower than levels reported by Flach (2003) and Nieminen and Timisjärvi (1983). Increased initial blood pressure, inhibition of vasopressin, and hyperglycemia from alpha-2 agonists, such as medetomidine, induce diuresis (Gellai and Edwards, 1988; Jalanka and Roeken, 1990), which may explain our decreased concentrations of potassium and phosphorus. Soveri et al. (1999) reported significantly decreased phosphorus, sodium, potassium, and chloride levels as compared with baseline values in captive reindeer within 60 min of administering medetomidine. Compared with Nieminen and Timisjärvi (1983), our mean value for free fatty acid is also low. A decrease in free fatty acid levels has been reported in captive reindeer following medetomidine injection (Soveri et al., 1999) and may be due to the inhibition of lipolysis by alpha-2 agonists (Taouis et al., 1988; Ambrisko and Hikasa, 2002).

Reindeer are sensitive to stress and increased levels of AST, cortisol, urea, and muscle lactate as well as depleted muscle glycogen stores have been demonstrated in stressed animals (Essén-Gustavasson and Rehbinder, 1984; Rehbinder, 1990). Cortisol increases occur with minimal handling as demonstrated by collection of blood in a zoo setting (Säkkinen et al., 2004), and medetomidine-induced increases in cortisol have already been mentioned. However, we found no correlation between induction time and stress parameters. Induction time and blood sampling may have occurred too quickly to see changes in blood results, or our limited sample size may have contributed too much variation to see a statistically significant relationship. Although the effects of drug combination, stress, season, and sample size should be considered when interpreting these values, we provide the first report of baseline hematologic and biochemical reference ranges for free-ranging, wild Norwegian reindeer during early winter.

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