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COPPER STATUS OF MUSKOXEN: A COMPARISON OF WILD AND CAPTIVE POPULATIONS

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ABSTRACT: We compared wild muskoxen (*Ovibos moschatus*) on Banks Island (Northwest Territories, Canada) with captive animals maintained on grass (*Bromus* sp.) hay and supplemental minerals. We measured copper (Cu) in liver, whole serum, and deproteinated serum (unbound Cu) as well as serum activity of the Cu-enzyme ceruloplasmin. Unbound serum Cu concentrations did not change with season in captive animals ($n=53$). Ceruloplasmin activity was similar between seasons in females but elevated in males during breeding in autumn. Increasing concentrations of Cu in whole sera were mainly associated with protein whereas unbound Cu predominated at low concentrations of whole serum Cu. Ceruloplasmin activity and serum Cu concentration were linearly related to liver Cu in female muskoxen. Measures of copper status in females were lower in the wild ($n=19$) than in captivity ($n=16$): 8 vs. 160 ug $Cu \cdot g^{-1}$ of whole liver; 0.67 vs. 1.15 μg unbound $Cu \cdot ml^{-1}$ whole serum and; 22 vs. 33 $IU \cdot l^{-1}$ ceruloplasmin activity. Bioavailability of Cu may limit the population on Banks Island especially when density of animals is high. The wide range of hepatic Cu concentrations in muskoxen indicated accumulation of Cu without apparent ill effect in captive animals. Hepatic storage of Cu may allow wild muskoxen to contend with low and fluctuating availability of Cu in small foraging areas at high latitudes.

Key words: Ceruloplasmin, deficiency, density dependence, heavy metals, *Ovibos moschatus*, ruminant, toxicity, trace minerals.

INTRODUCTION

Populations of arctic ungulates are constrained by extremes of winter weather (Forchhammer and Boertman, 1993; Mech, 2000) and by short summer plant production at high latitudes (Olesen et al., 1991). Muskoxen (*Ovibos moschatus*) may be particularly vulnerable to limited availability and quality of forages in the small home range occupied by each herd (Reynolds, 1998; Holm et al., 1999). Winter foraging may be restricted to stony terrain with low snow cover (Nelleman, 1997; Nelleman and Reynolds, 1997) whereas summer foraging may be limited by high density of animals (Raillard and Svoboda, 2000). Forage abundance and quality are greatest in summer (Klein and Bay, 1990; Oakes et al., 1992; Larter and Nagy, 1997; Ohlson and Staaland, 2001) when energy and nutrient demands are also high for growth, lactation, and restoration of mass lost in winter (Parker et al., 1990; Forchhammer, 1995; Forchhammer and Boomsma, 1995). Minerals required by herbivores are derived from plant tissues but deposition of minerals in plants is ultimately limited by quality of the soils and the underlying geology of the region (Van Soest, 1994). Forage production and geologic character of small home ranges probably limit supplies of minerals such as Na for muskoxen in Greenland (Thing et al., 1987; Forchhammer, 1995).

Trace minerals may also limit populations of herbivores at high latitudes; for example, lesions associated with copper (Cu) deficiency have been reported in moose from Alaska (Flynn et al., 1977; O'Hara et al., 2001) and Sweden (Frank et al., 1994). Interactions between trace minerals may also produce secondary aberrations; for example, absorption of dietary Cu may be reduced by elevated molybdenum (Mo) or by zinc (Zn; Robbins, 1993). Trace minerals may also limit growth and reproduction before signs of deficiency are apparent. Increased production and survival of young black-tailed deer (*Odocoileus hem-*

ionus) following supplementation of adults with selenium (Se) (Flueck, 1994) indicated inadequate Se for reproduction even though levels were apparently adequate for adult survival. Similarly, inadequate Cu from the diet or from tissue stores may increase the risk of mortality by reducing immune responses in humans, rodents (Failla and Hopkins, 1998), birds (Koh et al., 1996), and ruminants (Saker et al., 1998; Minatel and Carfagnini, 2000).

Most Cu is stored in protein complexes such as metallothionein within the liver (Cousins, 1985). Copper is circulated as ions and as protein complexes. Protein-bound Cu includes albumin, which transports Cu from the digestive tract to the liver, and ceruloplasmin, which exports Cu from the liver to tissues (Symonds and Forbes, 1993; Linder et al., 1998). The relationship between circulating forms of Cu and hepatic stores may be an important tool for evaluating Cu status when sampling the liver is not feasible (Barboza and Blake, 2001). Kinetics of serum ceruloplasmin in muskoxen include high reaction velocities and substrate affinities that may provide a wide range for monitoring changes in circulating Cu and hepatic storage (Barboza and Blake, 2001).

We compared wild muskoxen from Banks Island (Northwest Territories, Canada) with captive animals from Fairbanks (Alaska, USA). We measured serum Cu and ceruloplasmin in captive males and females through the year to assess stability of each measure when food was provided ad libitum with supplemental minerals. Serum Cu concentration was divided between ionic (unbound) and protein-bound fractions to describe their relationship with total extracellular Cu in circulation. Finally, we compared measures of circulating Cu with hepatic concentration of Cu to derive a predictive index of hepatic reserves in wild and captive animals.

MATERIALS AND METHODS

Captive animals

Studies of captive animals were authorized by the Institutional Animal Care and Use Committee, University of Alaska Fairbanks (Study # 98-039). The herd at the Institute of Arctic Biology (65°N, 146°W) was founded in 1980 from a population on Nunivak Island, Alaska, which was originally started with animals translocated from Greenland.

Captive muskoxen were fed grass hay (*Bromus* sp.) ad libitum, and a ration of pelleted food supplemented with minerals (Quality Textured Ration, Alaska Pet and Garden, Anchorage, Alaska) throughout the year. Minor amounts of fresh grass, forbs, and willow (*Salix* sp.) were also available in the summer. Supplement was provided in individual bins to each animal at 70 $g \cdot kg^{-0.75}$ each week in two to three equal portions. Supplement and hay were similar in concentrations of crude protein (19% of dry mass [DM]) and ash (6–8% DM) whereas concentrations of neutral detergent fiber were lower in the supplement than in the hay (18 vs. 49% DM). Mineral composition of DM in hay was typically 0.5% calcium (Ca), 0.2% phosphorus (P), 0.2% magnesium (Mg), 0.1% sulfur (S), 6 $\mu g \cdot g^{-1}$ Cu, 115 $\mu g \cdot g^{-1}$ iron (Fe), and 16 $\mu g \cdot g^{-1}$ Zn. The supplement typically contained 1.4% Ca, 1.1% P, 0.5% Mg, 0.3% S, 39 $\mu g \cdot g^{-1}$ Cu, 340 $\mu g \cdot g^{-1}$ Fe, and 140 $\mu g \cdot g^{-1}$ Zn. Feeds were low in Mo (<1 $\mu g \cdot g^{-1}$ DM) and therefore unlikely to form thiomolybdate complexes with Cu (Suttle, 1991). Final concentrations of nutrients can be calculated on the assumption that intakes of dry food are similar to those reported by Adamczewski et al. (1994) for captive muskoxen in winter (41 $g \cdot kg^{-0.75} \cdot day^{-1}$) and summer (62 $g \cdot kg^{-0.75} \cdot day^{-1}$). On this basis, an animal weighing 250 kg would receive 0.63 $kg \cdot day^{-1}$ supplement and would consume 2.6 $kg \cdot day^{-1}$ and 3.9 $kg \cdot day^{-1}$ of hay in winter and summer respectively. Dry mass of the final diet would therefore consist of 19% crude protein and 43% neutral detergent fiber with 0.6% Ca, 0.4% P, 0.2% Mg, 0.2% S, 13 $\mu g \cdot g^{-1}$ Cu, 162 $\mu g \cdot g^{-1}$ Fe, and 42 $\mu g \cdot g^{-1}$ Zn.

Seasonal changes in serum were assessed in two sets of samples ($n=53$): those collected throughout the year and archived from 1993–97 and those collected from breeding animals in 1998–99. Samples were archived during routine veterinary testing of 37 muskoxen >1 yr of age and in healthy condition. Sixteen adults (>4 yr old) were studied during the breeding season in August 1998 (four males and four castrated males), early gestation in December 1998 (four females), and in late gestation during February 1999 (four females). Although all females were exposed to bulls, one female did not have a fetus in February 1999. Blood was collected from the jugular vein into evacuated tubes without additive (Vacutainer Systems, Becton Dickinson, Franklin Lakes, New Jersey,

USA) centrifuged at 1,000 × G for 10 min, and stored at −70 C.

Serum was collected from 16 breeding animals in 1998–99 prior to euthanasia and necropsy. Animals were immobilized with a combination of xylazine (0.5 $mg \cdot kg^{-1}$; Xyla-ject®, 100 $mg \cdot mL^{-1}$, Phoenix Pharmaceutical, St. Joseph, Missouri, USA) and ketamine (5.5 $mg \cdot kg^{-1}$; Ketaject®, 100 $mg \cdot mL^{-1}$, Phoenix Pharmaceutical) or with a mixture of tiletamine and zolazepam (3.0 $mg \cdot kg^{-1}$; Telazol® 100 $mg \cdot mL^{-1}$, Fort Dodge Animal Health, Fort Dodge, Iowa, USA). Animals were euthanized by barbiturate overdose (12 ml containing 390 $mg \cdot ml^{-1}$ pentobarbital sodium and 50 $mg \cdot ml^{-1}$ phenytoin sodium, Euthasol®, Delmarva Laboratories, Midlothian, Virginia, USA). Liver was sampled at the caudate lobe within 60 min postmortem and stored at −20 C for analysis. All captive muskoxen used in this study were maintained under the UAF Animal Care Program and were fully evaluated postmortem by the university veterinarian to confirm their disease free status.

Wild animals

Liver and serum samples were collected from wild muskoxen (10 males >1 yr and 10 females >2 yr) on Banks Island during a harvest by Inuvialuit Peoples in November 1997. Age was estimated by size and shape of horns, body size, and pelage (Olesen and Thing, 1989). Harvest was by an Inuvialuit hunter with a single shot to the base of the skull with a large caliber rifle. Serum was collected directly into evacuated glass tubes from the jugular and allowed to separate for 2–4 hr at approximately 5 C before decanting serum into plastic vials for storage at −20 C. Liver was sampled at the caudate lobe and frozen for storage at −20 C. All samples were shipped frozen to University of Alaska Fairbanks for analysis. Sampling of wild muskoxen was limited to healthy animals that had passed inspection by an Agriculture Canada veterinarian approving the carcass for export and human consumption.

Chemical analysis

Ceruloplasmin was assayed by oxidase activity of 0.05 ml serum by modification of the o-dianisidine method (Schosinsky et al., 1974; Barboza and Blake, 2001). Protein was precipitated from 1.0 ml of serum by addition of 2.0 ml of 1.4 M HCl and 2.0 ml of 1.23 M trichloroacetic acid followed by centrifugation at 1,000 × G. Serum supernatant was below detectable limits for protein (<40 ug/ml) by the Bradford reaction (Sigma Chemicals, St. Louis, Missouri, Kit # B6916). Serum supernatant was analyzed by atomic-absorption spectrometry (AA) for Cu at 324.8 nm and Zn at 214 nm (Model 5000, PerkinElmer, Shelton, Connecticut, USA). This procedure retains ionic Cu and Zn in the supernatant but also includes minerals that were weakly bound to proteins or labile to acid. This fraction of unbound Cu and Zn is commonly used to evaluate serum Cu and Zn in ruminants (Melton et al., 1990; Barboza and Blake, 2001). Total Cu and Zn was determined by acid digestion of 1 ml serum and 1–2 g whole liver (Barboza and Blake, 2001). Digests were diluted with distilled, deionized water and assayed by AA against standards prepared from certified stock solutions (Fisher Scientific, Fairlawn, New Jersey). Liver samples were dried at 55 C to constant mass in a fan-forced convection oven to measure moisture content.

Calculations and statistics

Mineral concentrations were expressed on the basis of whole liver tissue by multiplying DM concentration by the content of DM in tissue ($gDM \cdot g^{-1}$ whole liver). Mineral concentrations were expressed as $\mu g \cdot g^{-1}$ whole liver tissue or $\mu g \cdot ml^{-1}$ whole serum. Coefficients of variation for replicate assays of minerals were 6% for serum and 10% for liver. Detection limits were 0.44 $\mu g\ Cu \cdot ml^{-1}$ and 0.11 $\mu g\ Zn \cdot ml^{-1}$ for serum and 0.97 $\mu gCu \cdot g^{-1}$ and 0.24 $\mu g\ Zn \cdot g^{-1}$ for liver. Concentrations of protein-bound minerals in serum were determined by difference between total and unbound concentrations. Ceruloplasmin activity was expressed as $IU \cdot l^{-1}$ ($mmol \cdot min^{-1} \cdot l^{-1}$) and is equivalent to 0.2863 $mg \cdot dl^{-1}$ of ceruloplasmin in whole human sera (Schosinsky et al., 1974). Minimum detection by this method is 1 $IU \cdot l^{-1}$ with 7% coefficient of variation among replicate assays.

Comparisons of ceruloplasmin activity between seasons and sexes were performed by analysis of variance (ANOVA) where seasons were divided as follows: early winter=October to January, late winter=February to April, spring and summer=May to July, and autumn=August to September. These periods correspond to the annual reproductive cycle, that is, breeding or rut in autumn, gestation through winter, and parturition at the start of spring. Castrates were combined with intact males in all comparisons because the two groups of males were similar for these measures ($P>0.05$). Pairwise contrasts between seasons were performed with Bonferroni's adjustments for multiple comparisons.

Relationships between measures of serum and liver were determined by linear least squares regression (Wilkinson and Coward,

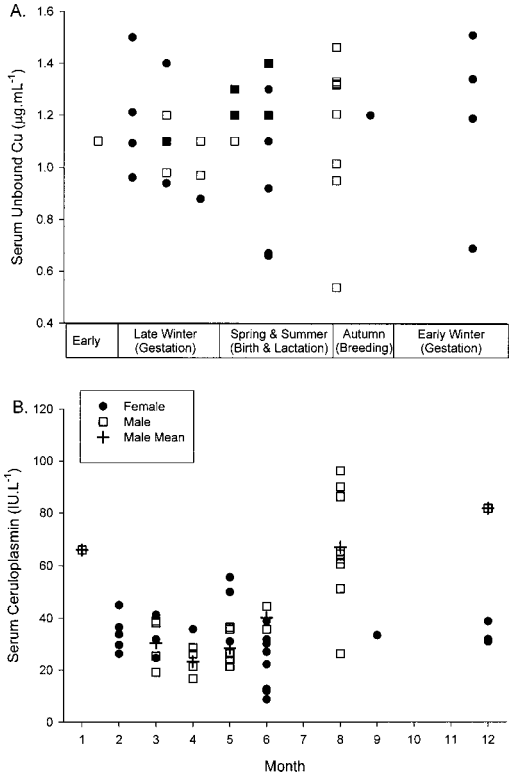

FIGURE 1. Seasonal changes of serum unbound Cu (A; $\mu g \cdot ml^{-1}$) and ceruloplasmin activity (B; $IU \cdot L^{-1}$) in male (open squares; A $n=23$; B $n=27$) and female (closed circles; $n=26$) muskoxen in captivity. Early winter (gestation)=October to January; late winter (gestation)=February to April; spring and summer (birth and lactation)=May to July; autumn (breeding)=August and September. Sexes and seasons were similar for unbound Cu ($P>0.05$). Mean activity of ceruloplasmin in males was greater in autumn than in late winter and spring ($P=0.003$). Ceruloplasmin activity of females was similar between seasons.

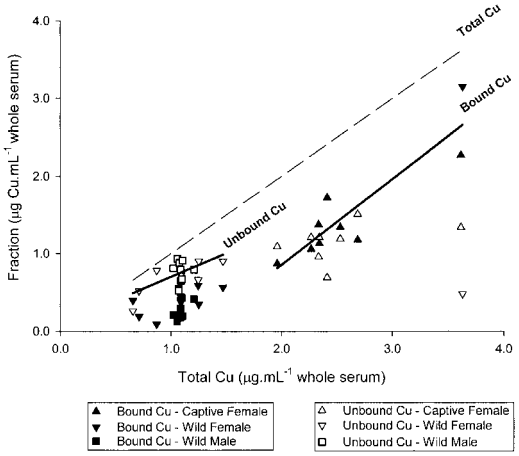

FIGURE 2. Distribution of Cu bound to proteins (closed symbols) and unbound acid-labile Cu (open symbols) in the serum of captive and wild muskoxen. The dashed line indicates complete correspondence with total Cu in serum. Solid lines indicate relationships between total Cu at low (<1.8 $\mu g \cdot ml^{-1}$) and high (>1.8 $\mu g \cdot ml^{-1}$) ranges with unbound Cu and with bound Cu respectively. Total $<1.8=0.60 \times$ unbound $+ 0.10 \pm 0.14$; $R^2=0.37$; $P<0.01$. Total$>1.8=1.11 \times$ bound $- 1.37 \pm 0.34$; $R^2=0.79$; $P<0.001$.

1998a). Regressions are reported with the standard error of the estimate (±SEE) and the square of the correlation coefficient (R^2) adjusted for multiple independent variables. Measures of serum and liver were compared by ANOVA for the effect of population, sex, and their interaction (Wilkinson and Coward, 1998b). Proportions were transformed to the arcsine of the square root to meet assumptions of normality for ANOVA (Zar, 1974). Statistical significance was determined as less than 5% probability of type I error ($P<0.05$). All means are reported with one standard deviation (±SD).

RESULTS

Unbound serum Cu did not vary with season in captive muskoxen (Fig. 1a). Although concentrations of Zn in serum varied from 0.4–1.1 $\mu g \cdot ml^{-1}$, those levels were similar between seasons and sexes ($P>0.05$). Ceruloplasmin activity of females was also unaffected by season, but activity increased in males during the rut in autumn (Fig. 1b).

Serum Cu concentration was mostly associated with proteins such as ceruloplasmin as total serum Cu increased above 1.8 $\mu g \cdot ml^{-1}$ (Fig. 2). Conversely, lower concentrations of Cu were mostly associated with unbound or free ions of Cu in serum (Fig. 2). Unbound Cu was not significantly related ($P>0.05$) to high concentrations of serum Cu (<1.8 $\mu g \cdot ml^{-1}$), while bound Cu was not related to low concentrations of serum Cu (>1.8 $\mu g \cdot ml^{-1}$).

Concentrations of unbound Cu in serum from female muskoxen were associated with activities of ceruloplasmin that were lower in the wild (22.0 ± 9.6 $IU \cdot L^{-1}$)

TABLE 1. Composition of serum and liver of male and female muskoxen in captivity at Fairbanks, Alaska and in the wild on Banks Island, Canada (mean ± SD). Liver dry matter is expressed as a proportion of whole tissue. Mineral concentration is expressed on the basis of whole liver and serum, respectively. Different superscripts within each row indicate significant between groups ($P < 0.05$).

Measure	Captive male (n = 8)	Wild male (n = 10)	Captive female (n = 8)	Wild female (n = 9)
Liver dry matter (% whole tissue)	31.1 ± 1.1	38.4^b ± 4.5	31.8^a ± 1.0	40.8^b ± 1.3
Liver Cu ($\mu g g^{-1}$)	195 ± 109	6.5^b ± 1.7	161^a ± 47	7.6^b ± 4.7
Liver Zn ($\mu g g^{-1}$)	69^a ± 43	18^b ± 28	56^a ± 11	116^c ± 18
Serum unbound Cu ($\mu g ml^{-1}$)	1.14^a ± 0.30	0.79^b ± 0.01	1.15^a ± 0.25	0.67^b ± 0.22
Serum unbound Zn ($\mu g ml^{-1}$)	0.79^a ± 0.11	0.91^b ± 0.19	0.87^a ± 0.12	0.77^a ± 0.17

than in captivity (33.5±5.9 IU·l^{-1}; Table 1; Fig. 3). Male muskoxen were excluded from regressions with ceruloplasmin activity because activity was elevated in captive males (Fig. 1b) even though liver Cu was similar to females in the captive population (Table 1). Circulating Cu was also related to the hepatic concentration of Cu in females (Fig. 3). This relationship predicts that liver Cu is exhausted at 0.7 $\mu g \cdot ml^{-1}$ for unbound serum Cu and at a ceruloplasmin activity of 7.5 IU·L^{-1} (Fig. 3). The relationship between liver Cu concentration, ceruloplasmin activity, and total serum Cu concentration was also linear: liver Cu=4.68 × ceruloplasmin + 42.6 × total serum Cu −129.28±58.96; R^2=0.52; P<0.01. This relationship predicts zero liver Cu concentration below 2.1 $\mu g \cdot ml^{-1}$ total serum Cu when ceruloplasmin activity is 8.5 IU·l^{-1}.

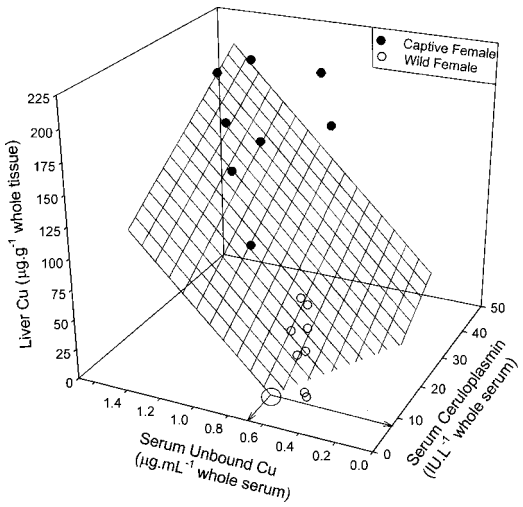

FIGURE 3. Relationship between serum ceruloplasmin activity (IU·l^{-1} whole serum), unbound acid-labile Cu ($\mu g \cdot ml^{-1}$ whole serum) and liver Cu ($\mu g \cdot g^{-1}$ whole tissue) of female muskoxen in captivity (solid circles) and in the wild (open circles). Liver Cu=2.51 × serum ceruloplasmin + 136.62 serum Cu − 114.52±64.4; R^2=0.43; P<0.05. The circle and arrows in the horizontal plane indicate minimal measures of serum that correspond to zero liver Cu concentration.

Although concentrations of Cu in serum and liver were lower in the wild than in captivity, levels of Zn in serum were similar (Table 1). Liver Zn concentration was greater in wild females than in captive females whereas the liver of wild males was lower in Zn than their captive counterparts (Table 1). Hepatic concentrations of Zn did not differ between sexes in captivity.

DISCUSSION

Accumulations of Cu in the liver are potentially toxic (Barboza and Vanselow, 1990; Eisler, 1997; Moriarty, 1999). Mean concentration of liver Cu in captive muskoxen (567 $\mu g \cdot g^{-1}$ DM) was higher than average values for domestic cattle (200 $\mu g \cdot g^{-1}$ DM; Underwood, 1977) and approached the level associated with toxicosis in sheep (1,000 $\mu g \cdot g^{-1}$ DM; National Research Council, 1980). Absence of clinical signs or lesions in our study animals suggests that muskoxen can sequester high concentrations of Cu without ill effects. Fetal and neonatal muskoxen also accumulate Cu in the liver (Rombach et al., 2002, 2003). These high concentrations are associated with the nuclear fraction of the rapidly dividing cells in other rumi-

nants (Leighton et al., 1990). Nuclear accumulation of Cu, however, is toxic in mature animals which primarily sequester Cu in the cytoplasm with proteins such as metallothionein and superoxide dismutase (Cousins, 1985). The mechanisms that allow mature muskoxen to tolerate high concentrations of Cu in the liver requires further study of hepatic proteins, cells, and tissues (Symonds and Forbes, 1993; Huffman and O'Halloran, 2001). Sequestration of large hepatic Cu reserves and high activities of ceruloplasmin in muskoxen may attenuate the effects of low and fluctuating availability of Cu in small foraging areas at high latitudes.

Although high liver Cu concentration was associated with an increase in protein-bound fraction of Cu in serum, enzyme activities are unlikely to reflect over abundance of Cu in circulation once sufficient Cu is available to saturate all active sites. Ceruloplasmin holds six atoms of Cu with high affinity, and nine more atoms on sites with lower affinities (Owen, 1982). Ceruloplasmin activity accounted for 43% of bound serum Cu in captive muskoxen with high levels of Cu in serum and liver. This estimate is based on the assumption that ceruloplasmin is 150,000 $g \cdot mol^{-1}$ and stocked with 15 atoms of Cu (Owen, 1982). Low levels of Cu in circulation may only provide Cu for sites on ceruloplasmin with high affinities. Ceruloplasmin activities at low Cu levels in wild muskoxen were equivalent to 68% of bound serum Cu if only six atoms are associated with each molecule of ceruloplasmin. Other Cu associated with protein was probably associated with albumen, transcuprein, and other oxidases (Wirth and Linder, 1985; Kehoe et al., 2000).

Metalloenzyme activities are also associated with metabolic changes that are not directly related to availability of metals for catalytic sites. Elevated activities of ceruloplasmin in captive male muskoxen were not related to high levels of Cu in the liver when compared with females in the same herd (Table 1) and were not due to lesions or infections. Behavioral changes associated with the breeding period in muskoxen (Forchhammer and Boomsma, 1998) probably induce elements of the acute-phase response which has been associated with elevated ceruloplasmin activity (Minatel and Carfagnini, 2000). This suggestion is supported by similar elevations of ceruloplasmin in male reindeer and caribou (*Rangifer tarandus*) after rut (Barboza and Blake, 2001) and by differences in ceruloplasmin activity between sexes in humans (Kehoe et al., 2000). The difference in ceruloplasmin activity between sexes of muskoxen is probably restricted to the breeding period in wild populations because males were similar to females in liver Cu (Table 1) and in ceruloplasmin activity (male 26.4 ± 7.9 $IU \cdot l^{-1}$ vs. female 22.0 ± 9.8 $IU \cdot l^{-1}$) during November (early winter) on Banks Island. Late winter measures of ceruloplasmin activity may also include a small depression of ceruloplasmin activity in pregnant females. Repeated measures of ceruloplasmin activity during gestation indicates a small decline in activity during peak fetal development in muskoxen (Rombach et al., 2003).

The combination of serum Cu concentration and ceruloplasmin activity can provide an index of low liver Cu concentration in muskoxen (Fig. 3) when repeated measures are required or when necropsy is not feasible. Although the lower critical limit of circulating Cu concentration is unknown for muskoxen, the criteria of 0.7 $\mu g \cdot ml^{-1}$ unbound serum Cu and 7.5 $IU \cdot l^{-1}$ ceruloplasmin could be used for monitoring low liver Cu before specific signs of deficiency arise in populations.

Circulating and hepatic Cu concentrations probably vary between populations of muskoxen. Serum Cu in muskoxen on Banks Island in 1997 (mean=0.73 $\mu g \cdot ml^{-1}$) and 1985 (mean=0.78 $\mu g \cdot ml^{-1}$; unpubl. data of Blake and Rowell in Blakley et al., 1998) are lower than those reported from Victoria Island (Nunavut, Canada) in 1995 (mean=0.92 $\mu g \cdot ml^{-1}$; Blakley et al., 2000). Mean liver Cu con-

centration declined from 68.3 to 16.9 $\mu g \cdot g^{-1}$ on Victoria Island between 1989 and 1995 and from 29.9 to 7.0 $\mu g \cdot g^{-1}$ on Banks Island between 1985 and 1997 (Salisbury et al., 1992; unpubl. data of Blake and Rowell in Blakley et al., 1998; Blakley et al., 2000). Populations of muskoxen on Banks Island more than doubled between 1985 and 1994 but subsequently declined by 30% in just 4 yr (Larter and Nagy, 2001). Copper availability may be associated with the population decline because levels of liver Cu in adult muskoxen during 1997 (17 $\mu g \cdot g^{-1}$ DM) are below the criterion for deficiency in domestic cattle (20 $\mu g \cdot g^{-1}$ DM; National Research Council, 2000). This population decline was mostly associated with low survival and recruitment of calves but not with mortality of adults (Larter and Nagy, 2001). Low liver Cu concentration in pregnant females can limit deposition of Cu in the fetus (Symonds and Forbes, 1993) which may compromise development in utero (Lee et al., 2001). Low fetal reserves of Cu may subsequently compromise neonatal immunity and growth because milk of ruminants including muskoxen is low in Cu (Grace and Clark, 1991; Rombach et al., 2002). This possibility awaits further confirmation from studies of Cu and immunity in developing muskoxen.

Seasonal changes in foraging behavior probably affects mineral balance and hepatic reserves of muskoxen in the wild (Staaland and Thing, 1991). Differences between the sexes in hepatic Zn concentration in muskoxen from Banks Island (Table 1) probably reflect differences in food selection and movement between sexes (Gunn et al., 1989; Smith, 1989; Oakes et al., 1992). Low hepatic Cu concentration in both sexes of wild muskoxen is probably due to the low concentration of Cu in graminoid plants (National Research Council, 2000) that constitute the bulk of their diet on Banks Island (Larter and Nagy, 1997). Concentrations of Cu in forbs and legumes can exceed those of graminoids (National Research Council, 2000) and provide a supplemental source of minerals. For example, arctic sedges and grasses contain 2–10 μg Cu$\cdot g^{-1}$ DM whereas louse-worts, willows, and herbs may contain up to 15 μg Cu$\cdot g^{-1}$ DM (Staaland and White, 2001). Bioavailability of Cu from these non-graminoids may be reduced by other dietary components such as Mo, S, and alkaloids (National Research Council, 2000). Furthermore, this potential source of Cu may be diminished by overutilization of favored plants (Mulder and Harmsen, 1995) especially at high densities of animals. Copper supply may be one index of density dependence effects on muskoxen, that is, depletion of the abundance and diversity of forage could reduce Cu availability. Captive muskoxen can double food intakes between April and September. These high intakes of graminoids that are low in Cu (<5 $\mu g \cdot g^{-1}$ DM) may allow growth and sufficient storage of Cu in the liver for the ensuing winter (Peltier and Barboza, 2003; Peltier et al., 2003). Demands for Cu in immunologic responses would increase as the frequency of exposure to pathogens such as parasites increases with population density (Sinclair, 1997). The hypothetical interaction between population density, nutrition, and immunity in herbivores at high latitudes need further testing through measures of foraging behavior, trace nutrient status, and population dynamics.

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