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TRACE MINERAL AND VITAMIN CONCENTRATIONS IN THE LIVER AND SERUM OF WILD MUSKOXEN FROM VICTORIA ISLAND

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ABSTRACT: Selected trace minerals and vitamins were assayed in the liver and serum of 25 wild muskoxen (*Ovibos moschatus*) from Victoria Island, (Nunavut, Canada) in November, 1995. Mean ± SE liver concentrations in μmol/kg wet weight were 260 ± 16 for copper; 1.04 ± 0.06 for selenium; 11.5 ± 0.7 for molybdenum and 62.8 ± 3.3 for vitamin E. Mean ± SE serum concentrations in μmol/L were 14.2 ± 0.3 for copper; 0.75 ± 0.04 for selenium, 1.53 ± 0.07 for vitamin A and 5.80 ± 0.55 for vitamin E. Comparison of liver and serum concentrations of copper, selenium and vitamin E showed that the concentration in one tissue was a relatively poor indicator of the concentration in the other. The copper-molybdenum interaction often seen in domestic species was not observed. In general, the concentrations of metals and vitamins found in muskoxen were comparable to those in other ungulates although serum vitamin E concentrations were about one-fourth of those expected.

Key words: Copper, molybdenum, muskox, nutrition, *Ovibos moschatus,* selenium, vitamin A, vitamin E.

INTRODUCTION

Trace nutrient concentrations have been studied extensively in the blood and tissues of domestic animals for physiological, nutritional and diagnostic purposes. In contrast, there is little comparable information on wildlife, and extrapolation from domestic species may be unreliable for either physiological or environmental reasons.

Muskoxen (*Ovibos moschatus*) inhabit the North American tundra where there is no plant growth for about 9 mo of the year; the soils are poor, and the climate is extremely harsh. Such conditions may affect the concentrations of trace nutrients in the serum and liver. Studies on muskox micronutrients are limited but there are reports on trace metals (Blake and Rowell, 1985; Salisbury et al., 1992; Gamberg and Scheuhammer, 1994) and vitamins A and E (Ghebremeskel and Williams, 1988).

Confined muskoxen, outside their natural range, may have different tissue micronutrient levels from their wild counterparts because of differences in nutrition and environment. More information on the vitamin and trace mineral status of healthy wild populations is required to fully understand the nutrition and health of both wild and captive animals. In this study we make use of hunter-killed muskoxen on Victoria Island to examine the levels of copper, molybdenum, selenium, and vitamins A and E in a sample of apparently normal muskoxen.

MATERIALS AND METHODS

The study population and area

In 1995 the muskox population of Victoria Island was about 45,000 (Fournier and Gunn, 1998) and had increased greatly since the 1960's (Gunn, 1990). The terrain occupied by the animals is a polar semi-desert with a continental climate, and comprises rolling tundra interspersed with rocky areas, lakes and eskers. It is predominantly glacial till, with a high clay and limestone content, and low hills of quartzite and sandstone. The vegetation is dominated by graminoids, *Dryas* spp. and *Salix* spp. and includes moist meadows and some erect shrubs (Schaefer and Messier, 1994). Much of the ground is snow covered from late September to late June. At the time of sample collection all available forage was dead or dormant.

Post-mortem examination by a veterinarian (S. J. K.) showed the animals to have ample fat reserves and failed to reveal significant disease. Detailed studies of this population from 1989 to 1993 showed most animals to be in good body condition (Adamczewski et al., 1997) and from 1993 to 1999 there was a population in-

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crease of 7% annually (B. Patterson, pers. comm.).

Sample collection

Serum and liver samples were collected in mid-November, 1995. The muskoxen were shot by Inuit hunters near the Ekalluk River (69°22′N, 106°12′W) on southern Victoria Island (Nunavut, Canada), usually after a snowmobile chase of less than 5 min or 500 m. Normally, when a small herd was located, all its members were killed. Specimens were obtained from several such herds. Blood was collected from a jugular vein immediately after death and allowed to clot. The serum was separated by centrifugation each evening and the samples frozen. Most of the digestive tract was removed in the field but the liver was left in situ. Liver samples were collected and frozen (at outside ambient temperature) 1–4 hr after death when the carcasses arrived at the temporary field abattoir. Temperatures ranged from −20 C to −30 C during the collection. Muskoxen were assigned to the following age classes according to the appearance of their horns and teeth: 1.5, 2.5, 3.5 and ≥4.5 years (see Olesen and Thing, 1989). In total, samples were collected from six males (2.5 yr, $n = 3$; ≥4.5 yr, $n = 3$) and 18 females (1.5 yr, $n = 1$; 2.5 yr, $n = 5$; 3.5 yr, $n = 7$; ≥4.5 yr, $n = 5$). All assays were performed within 2 wk of collection.

Analysis of samples

Copper, selenium and vitamin E were measured in liver and serum, vitamin A in serum only and molybdenum in liver only. A liver vitamin A assay was not readily available for this study and serum molybdenum concentrations are beneath the limit of detection of our system. All analyses were performed at the Diagnostic Toxicology Laboratory Western College of Veterinary Medicine (Saskatoon, Saskatchewan, Canada).

Copper was assayed by standard spectroscopic techniques (Sunderman, 1975; Hyde et al., 1977). Serum samples (≥0.75 ml) were diluted to 2.0 ml with distilled deionized water and mixed with 2.0 ml of 1.4 M HCl. After 30 min, 2.0 ml of 1.23 M trichloroacetic acid was added to deproteinate the sample. The supernatant was analyzed on a Perkin-Elmer 2380 Atomic Absorption Spectrophotometer (Perkin-Elmer, Rexdale, Ontario, Canada) at a wavelength of 324.8 nm. The detection limit was less than 0.1 μmol/L.

Liver samples (approximately 2 g) were digested overnight in an acid mixture containing 5 ml concentrated nitric acid : 1 ml concentrated sulfuric acid : 1 ml concentrated perchloric acid (Salisbury and Chan, 1985), using a Tecator Digestor, Digestion System 20, equipped with a programmed temperature control, Autostep 1012, and a model 1013 scrubber unit (Tecator AB, Hoganas, Sweden). The residue was diluted with distilled-deionized water and analyzed on the spectrophotometer for copper. The detection limit for liver copper was 2 μmol/kg.

Serum selenium was measured using a flameless atomic absorption technique (Anonymous, 1983). Samples (0.1 ml) were diluted in 0.4 ml of a 1.0% Triton-X 100 diluent (VWR Scientific, Edmonton, Alberta, Canada). To this mixture, 20 μl of a 1% silver nitrate solution (0.093 M) prepared in 0.1 M nitric acid was added. This solution was added in 10 μl volumes to a tungsten filament (Seigniory Chemical Products Ltd., St. Laurent, Québec, Canada) in the burner compartment of a Scintrex Zeeman Modulated Atomic Absorption Spectrophotometer model AAZ-2 (Scintrex Ltd., Concord, Ontario, Canada) equipped with Zeeman background correction. The absorption of selenium was monitored at a wavelength of 196.0 nm. Argon gas was used to flush the atomized sample through the system. Under the stated conditions, the detection limit for selenium in serum was approximately 0.2 μmol/L.

Liver samples for selenium analysis were digested using the procedure described for copper (Salisbury and Chan, 1985). The digested samples were diluted to 50 ml with distilled-deionized water and analyzed on a Perkin-Elmer 2380 Atomic Absorption Spectrophotometer equipped with a hydride generator and a selenium EDL lamp at a wavelength of 196 nm (Anonymous, 1986). A portion of the diluted sample was acidified with sulfuric acid and reduced with sodium borohydride. The hydride derivative of selenium was flushed through the spectrophotometer using argon gas. The detection limit for selenium in the liver using the hydride method was approximately 0.01 μmol/kg.

For molybdenum, the liver was digested overnight as for copper analysis. Five ml of distilled-deionized water and 1 drop of methyl orange (1.0 mmol/L) were added to the digest residue and the solution was neutralized with concentrated ammonia. Five ml of 6 M HCl and 4 ml of 1.2 M sodium fluoride were then added, and the solution was transferred to a separator funnel and diluted to a 30 ml volume with distilled water. Four ml of 2.06 M potassium thiocyanate and 3 ml of 1.06 M stannous chloride were added with mixing and extracted with 7 ml of isoamyl alcohol. The alcohol layer was extracted with 25 ml of 0.16 M stannous

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chloride and centrifuged for 30 min at 2,000 rpm. The absorbance of the alcohol layer was measured on a UV-visible spectrophotometer (Beckman DU65, Beckman Instruments Inc., Fullerton, California, USA) at a wavelength of 465 nm. The detection limit for molybdenum in the liver was approximately 1.0 μmol/kg.

Serum vitamin A (all-trans retinol) and vitamin E (α-tocopherol) levels were determined by high pressure liquid chromatography (HPLC) with ultraviolet detection at 325 nm and 285 nm, respectively (Blakley and Bell, 1994) using α-tocopherol, α-tocopheryl acetate, all-trans retinol and all-trans retinol acetate as standards (Eastman Kodak, Rochester, New York, USA). Serum samples (2.0 ml) were mixed with 0.4 ml of the internal standards, the acetate derivatives of retinol or alpha-tocopherol, and 1.6 ml of absolute ethanol. The mixtures were extracted with an equal volume of petroleum ether (b.p. 30–60 C; Petroleum Spirit "OmniSolv"; BDH, Edmonton, Alberta, Canada). The petroleum ether phase was evaporated in darkness under nitrogen. The residue was dissolved in methanol (BDH, Edmonton, Alberta, Canada) and injected onto an HPLC column (Beckman ODS-ultrasphere 5 μm column, 4.6 × 15 cm, Beckman Instruments Inc., Fullerton, California, USA). The vitamins were eluted with a methanol:water mobile phase at a U:V ratio of 90:10 for vitamin A and 97:3 for vitamin E. The detection limits for vitamin A and vitamin E were 0.04 μmol/L and 0.5 μmol/L, respectively, in serum.

Liver vitamin E levels were determined with an HPLC method using ultraviolet detection at 285 nm (Taylor et al., 1976) and modifications of the serum chromatographic procedures. Liver (3 g) was homogenized in 25 ml of isotonic potassium chloride (0.146 mmol/L). One half ml of ascorbic acid (0.01 mmol/L) and 1.0 ml of absolute ethanol were added to the homogenate. The mixture was incubated at 70 C for 5 min. One ml of potassium hydroxide (10 mmol/L) was added to saponify the homogenate and incubated for 30 min at 70 C. The cooled mixture was extracted with 4.0 ml of petroleum ether. The ether layer was removed and prepared in a fashion similar to the serum samples for HPLC analysis. The reconstituted extract was analyzed using ultraviolet detection at a wavelength of 285 nm. The detection limit for vitamin E in the liver was approximately 1 μmol/kg.

Statistical analysis

Much of the data did not lend themselves to detailed statistical analysis owing to small numbers in some groups. Tissue comparisons and copper-molybdenum interactions were analyzed by simple regression.

RESULTS

Liver and serum concentrations of copper, selenium, molybdenum, vitamin E, and vitamin A are summarized in Table 1. Concentrations of micronutrients in the liver are expressed according to liver wet weight. Correlations between liver and serum concentrations of copper, selenium, and vitamin E were poor (Table 2). There was no correlation between concentrations of liver copper and liver molybdenum ($P = 0.16$), or serum copper and liver molybdenum ($P = 0.66$) (Table 3).

DISCUSSION

The mean concentration of selenium found in muskox livers in the present study (1.04 μmol/kg) was very close to that reported for the same area in April and May, 1989 ($\bar{x}$ = 1.27 μmol/kg; Salisbury et al., 1992). However, selenium concentrations in the livers of muskoxen killed on Banks Island in May, 1985 were four times ($\bar{x}$ = 455 μmol/kg; Blake and Rowell, 1985) those reported here. There are marked geological differences between Banks and Victoria Islands; Banks Island is part of the arctic coastal plane and consists mainly of non-marine sedimentary rocks. The concentrations found in muskoxen from Banks Island would be "adequate" for domestic cattle but the concentrations in muskoxen from Victoria Island would be "marginal" (Puls, 1994a).

The serum selenium concentrations reported in the present study (0.75 μmol/L) differed little from those for Banks Island muskoxen ($\bar{x}$ = 0.56 μmol/L; Blake and Rowell, 1985) and for confined animals in Saskatchewan ($\bar{x}$ = 1.06 μmol/L; Blake and Rowell, 1985). In domestic livestock, these concentrations would be "marginal" (Puls, 1994a; Osweiler et al., 1985).

Domestic animals differ in their selenium requirements, and selenium status varies widely depending on the concentrations in soil and plants (Osweiler et al.,

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TABLE 1. Micronutrient concentrations in liver (μmol/kg) and serum (μmol/kg) from wild muskoxen; mid-November 1995; Victoria Island, Nunavut, Canada.

		Male			Female			All		
Tissue	Analysis	Mean ± SE	Range	*n*	Mean ± SE	Range	*n*	Mean ± SE	Range	*n*
Liver	Copper	315 ± 91	120–710	6	244 ± 32	60–470	19	260 ± 16	60–710	25
	Selenium	1.10 ± 0.09	0.7–1.30	6	1.02 ± 0.08	0.2–1.4	19	1.04 ± 0.06	0.2–1.4	25
	Molybdenum	12.0 ± 1.2	9.3–16.9	6	11.4 ± 0.9	5.3–19.2	19	11.5 ± 0.7	5.3–19.2	25
	Vitamin E	60.5 ± 3.7	52.9–74.0	6	63.8 ± 4.2	36.5–107	19	62.8 ± 3.3	36.5–107	25
Serum	Copper	15.5 ± 0.6	14.0–18.1	6	13.8 ± 0.3	11.0–15.9	18	14.2 ± 0.3	11.0–18.1	24
	Selenium	0.80 ± 0.07	0.45–0.95	6	0.74 ± 0.05	0.47–1.11	18	0.75 ± 0.04	0.45–1.11	24
	Vitamin A	1.85 ± 0.11	1.48–2.10	6	1.42 ± 0.07	0.42–1.76	18	1.53 ± 0.07	0.42–2.10	24
	Vitamin E	5.40 ± 1.13	1.56–9.29	6	5.66 ± 0.63	1.46–10.5	18	5.80 ± 0.55	1.46–10.51	24

TABLE 2. Correlation between serum and liver concentrations of micronutrients in muskoxen; mid-November 1995; Victoria Island, Nunavut, Canada.

Analysis	Correlation coefficient	Number of pairs	Probability[a]
Copper	−0.022	24	0.92
Selenium	0.20	22	0.36
Vitamin E	−0.31	24	0.14

[a] Probability of no correlation between concentrations in serum and liver.

1985). Because selenium is important for the enzymic repair of oxidative damage, the availability of nutrients with direct antioxidant properties, especially vitamin E, affects the animal's response to selenium deficiency.

The mean liver copper concentration reported in the present study (260 μmol/kg) was one-quarter that found on the same area of Victoria Island in April and May 1989 ($\bar{x}$ = 1047 μmol/kg; Salisbury et al., 1992) and about half that for Banks Island in May 1985 ($\bar{x}$ = 455 μmol/kg; Blake and Rowell, 1985). Clearly the liver copper reserves of healthy muskoxen are quite variable. If this is a seasonal change the reason is obscure. Transfer of copper from mother to fetus would be expected to have the opposite effect and the liver was at its small "winter" size (Adamczewski et al., 1997) during all the collections. Copper, however, is more readily absorbed when forage is dry, when dietary protein is low and when gut transit time is long (Puls, 1994a). The fact that all these conditions are met by muskoxen during the arctic winter (Adamczewski et al., 1994) might explain the relatively high liver copper levels seen in April and May.

All but six of the liver copper concentrations reported here would lie in the range described as "marginal" had they been seen in cattle or sheep (Puls, 1994a). Of the remainder, four were "adequate" and two "deficient." Nevertheless, the range of serum copper concentrations reported here was similar to that of normal domestic ruminants (Puls, 1994a; Osweiler

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TABLE 3. Correlation between copper and molybdenum levels in the serum or liver in muskoxen; mid-November 1995; Victoria Island, Nunavut, Canada.

Analysis	Correlation coefficient	Number of pairs	Probability[a]
Liver copper with liver molybdenum	0.29	25	0.16
Serum copper with liver molybdenum	−0.094	24	0.66

[a] Probability of no correlation.

et al., 1985), wild muskoxen on Banks Island, and confined muskoxen in Saskatoon (Blake and Rowell, 1985).

Because the serum copper concentrations were in the normal range and there was no correlation between serum and liver concentrations, it appears that liver concentrations of 100–700 μmol/kg are compatible with physiological copper concentrations in the blood. Absence of a correlation between blood and liver copper concentrations would be anticipated in animals drawn from a single homogeneous population that is not obviously deficient (Blakley and Hamilton, 1985; Blakley et al., 1992).

Although both serum and liver copper concentrations were lower in females than males the effects were small and of doubtful biological or statistical significance. Nonetheless, liver copper levels of nonpregnant female wapiti (*Cervus elaphus*) have been found to be almost double those of their pregnant counterparts (Blakley et al., 1992), and liver copper concentrations decline with age in red deer (*Cervus elaphus*) (Wilson et al., 1979; Leighton et al., 1990). It seems likely that pregnancy can deplete copper reserves as a result of preferential transfer of copper to the fetus. This hypothesis is supported by the fact that the only animal to die when copper deficiency occurred in confined muskoxen was an aging multiparous female (Blakley et al., 1998).

The mean liver molybdenum concentration found in muskoxen in the present study was within the normal range for cattle, but slightly less than that for sheep (Puls, 1994a). Although an inverse relationship between copper and molybdenum has been reported in many parts of the world and a variety of species (Osweiler et al., 1985; Radostits et al., 1994), we found no evidence of such an interaction in our data. Furthermore, higher concentrations of both copper and molybdenum were present in muskox livers from Banks Island (May 1985; $\bar{x}$ = 455 μmol/kg for copper; $\bar{x}$ = 18.7 μmol/kg for molybdenum; Blake and Rowell, 1985) than from Victoria Island (November 1995; this study). Taken together, these observations suggest that the relationship between copper and molybdenum may be weaker in muskoxen than in cattle and sheep. On the other hand, the absence of an inverse correlation may merely mean that all the muskoxen studied to date have had molybdenum concentrations within tolerable limits. In domestic ruminants, copper availability is not impaired until hepatic molybdenum concentrations are at least twice those seen in this study (Osweiler et al., 1985).

Serum vitamin A concentrations for Victoria Island muskoxen (1.53 μmol/L) were very similar to those reported for healthy, domestic ruminants and only marginally less than the mean for three muskoxen kept in a zoo paddock in southern England (2.1 μmol/L; Ghebremeskel and Williams, 1988). Serum vitamin A levels were somewhat lower in females than males, perhaps because of lactation. In sheep and cattle, serum vitamin A concentrations increase from birth to adulthood (Puls, 1994b) but such an effect was unlikely to be detected in this study because it included only a single yearling; the other animals were all ≥2.5-yr-old.

Liver vitamin E concentrations were at the upper end of the range considered ad-

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equate for domestic ruminants. This is probably adaptive because it is unlikely that the muskox winter diet of weathered, dry, frozen herbage is a good source of vitamin E. In November, when the samples were collected, muskoxen face 8 mo without access to new growth. Like other species, muskoxen probably carry large, if less mobile, reserves of vitamin E in their fat in addition to that in the liver (Jensen et al., 1988). There was no significant correlation between serum and hepatic vitamin E concentrations. This is perhaps to be expected if body reserves exceed immediate requirements and dietary vitamin E is low.

The mean serum vitamin E concentration in Victoria Island muskoxen was about one-quarter of that in other well-nourished herbivores (Puls, 1994b) but slightly greater than that for three muskoxen kept in a zoo paddock in southern England (4.4 μmol/L; Ghebremeskel and Williams, 1988). It is unclear whether the exceptionally low serum vitamin E concentrations are a characteristic of this species or consequence of low dietary vitamin E at the time of sampling.

In ungulates, serum vitamin E concentrations are usually low in young rapidly growing animals and increase with maturity (Blakley and Bell, 1994; Puls, 1994b). As discussed in connection with vitamin A, the age distribution of the animals in this study is unlikely to reveal such an effect.

Vitamin E, like selenium, inhibits free radical oxidation of cell membranes. Deficiencies occur in ruminants fed poorly conserved rations and denied access to fresh pasture. As with vitamin A, the seasonal dynamics of vitamin E merit attention in wild muskoxen. Animals at the end of their first winter might be expected to be most at risk of vitamin E deficiency especially if selenium concentrations are also low.

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