Variation in Serum Ferritin, Serum Iron, Total Iron Binding Capacity (TIBC) and Percent Transferrin Saturation in Northern Fur Seals: a Retrospective Study

Lisa M. Mazzaro, J. Lawrence Dunn, David J. St. Aubin
Mystic Aquarium, 55 Coogan Blvd., Mystic, CT 06355

Gordon A. Andrews, Patricia S. Chavey
Kansas State Veterinary Medical Center, 1800 Denison Ave., Manhattan, KS 66506

Hemochromatosis is an excessive accumulation of iron in tissues, particularly liver, and is associated with the development of hepatic lesions. The term hemosiderosis is used when there are no toxic effects of the iron accumulation. Both conditions have been observed in free-ranging and captive specimens of many species (Lowensteine and Munson 1999). Histopathologic analysis of tissues obtained from two aged female northern fur seals at Mystic Aquarium demonstrated large amounts of iron in the liver. This study examines whether changes in four analytes (serum ferritin, serum iron, TIBC and percent saturation) over time might signal iron storage problems. In humans, serum ferritin determination is second only to liver biopsy as an indicator of liver iron levels. Ferritin analysis may be more difficult in other species because this analyte may be species specific.

Mystic Aquarium is one of only three facilities listed in the northern fur seal stud book and holds the largest breeding colony of these animals with the widest range of age groups. One hundred banked serum samples from 25 fur seals (17 females and 8 males) held at Mystic Aquarium over the past 11 years (1989-1999) were examined to determine serum iron analytes and their association with sex, age and genetic relationship. Animals ranged in age from less than 1 year to an estimated > 22 years. Data from pups (<2 years old) of both sexes were combined. Males were divided into 3 age groups; juveniles (2-5 years), sub-adults (6-9 years) and adults (>9 years). Different age groupings were established for females because they achieve full body size at an earlier age and because they tend to have longer lifespans than males. Juvenile females were 2 to 5 years, adults 6 to 17 years and geriatric females were those greater than 17 years.

Serum ferritin was measured for the first time in this species using an ELISA assay incorporating monoclonal anti-canine ferritin antibody and fur seal ferritin as a standard. Serum iron and TIBC were measured coulometrically using an ESA Ferrochem II iron analyzer.

Comparison by sex regardless of age showed males to have significantly lower serum iron, percent saturation and ferritin, and higher TIBC than females in this study. Each sex was also considered separately for determination of age related differences (Table 1). Serum ferritin showed a positive correlation with age in females. Percent saturation was less and TIBC greater in juveniles than in adults and geriatric females. Among males there were no significant age differences in any of the analytes.

Adult males had significantly lower percent saturation and less serum ferritin than adult females.

Data were analyzed for relationships between parameters. A positive correlation exists between age and percent transferrin saturation ($r = 0.44$, $p < .0001$) and between age and serum ferritin ($r = 0.74$, $p < .0001$). A negative correlation between age and TIBC ($r = -0.66$, $P < .0001$) was also found. The data also showed a relationship among the eight mother/pup pairs. Serum iron ($r = 0.83$, $p < .05$) and percent transferrin saturation ($r = 0.80$, $p < .05$) were correlated suggesting that the pups may be showing iron levels due to placental transfer of iron and/or transfer through the milk from iron overloaded females. Iron levels in pups decreased after weaning.

Table 1. Serum iron analytes (mean +/- SD) in clinically normal northern fur seals broken down by age and sex.

Serum analyte N=	**Pups (both)** 5 month– 2yr (10)	**Juvenile Male** 2-5 years (10)	**Sub-Adult Male** 6-9 years (14)	**Adult Male** >9 years (6)	**Juvenile Female** 2-5 years (11)	**Adult Female** 6-17 years (27)	**Geriatric Female** >17 years (14)
Iron (ug/dl)	149 +/- 72.9	98 +/- 30.5	85 +/- 30.6	85 +/- 28.2	96 +/- 47.0	112 +/- 38.8	105 +/- 24.2
TIBC (ug/dl)	304 +/- 47.5	274 +/- 87.9	242 +/- 45.7	225 +/- 38.0	279 +/- 79.1	180 +/- 33.1	178 +/- 25.2
Saturation (%)	49 +/- 22.4	37 +/- 8.8	34 +/- 7.9	39 +/- 13.7	36 +/- 17.0	62 +/- 14.0	59 +/- 6.5
Ferritin (ng/ml)	15 +/- 11.2	37 +/- 35.8	43 +/- 35.1	65 +/- 41.2	26 +/- 19.1	371 +/- 295.5	713 +/- 317.4

Note- only 92 samples are included in this table. Some were omitted because they were taken from animals close to time of death and upon necropsy diagnosed with hemochromotosis and other were from pups less than 5 months of age and still nursing. This data will be shown in table form at the conference and in the full paper.

Diet was considered as a factor in the development of iron storage disorders in northern fur seals. It is very difficult however to compare the captive dietary iron levels with that of the natural diet, which is quite diverse and varies with location, season, and year, among other factors. In the wild, the most common prey are Walleye pollock and gonatid squid; Pacific herring and capelin are encountered much less frequently. By contrast, the diet of this colony of captive northern fur seals prior to 1998 consisted of herring (usually 50% or more), capelin (15 to 20%), squid (0 to 15%) and occasionally mackerel. This diet provided 2.5 to 5 mg iron per kg body weight. Two dietary changes were made in 1998: the proportion of squid was increased to 15-45% and the herring component decreased, and a multivitamin supplement containing iron (12 mg/tablet) was replaced with an iron free multivitamin. On a mg/kg diet basis iron intake decreased between 19 and 47% and on a mg/kg body weight basis decreased between 11 and 36%. When pre (1998) and post (1999) dietary change sera were analyzed with a paired t-test no significant changes in any of the iron-related anyalytes were found. Possible explanations

for this lack of expected changes are 1) decreasing dietary intake may not lower body iron (phlebotomy or iron chelating drugs may be necessary), 2) the dietary decrease (although significant) was not enough to have any effect, 3) not enough time had passed to see any effect or 4) efficiency of iron absorption increased as dietary iron decreased. In addition both multivitamins contain vitamin C which is known to enhance iron absorption.

In summary our results show that there are both sex and age differences in serum iron analytes of northern fur seals. The trend is toward increased serum ferritin and percent transferrin saturation with age especially in the females. In humans, hemochromatosis is more prevalent in males whereas in the northern fur seals only females have been diagnosed with this condition to date. Possible causes of hemochromatosis include inborn errors, excessive parenteral administration and high oral intake. In the case of the northern fur seals we suggest that the increased dietary iron resulting from a higher proportion of fish over squid in the diet, as compared to wild fur seal diets, as well as iron and vitamin C supplementation have played a role in the development of this disease. With improved care, captive animals are living longer than their wild counterparts, perhaps allowing greater opportunity for such conditions to develop. As this colony of fur seals ages we must explore additional avenues to find a methods to lower liver and body iron levels.

References

Lowensteine, L.J. and L. Munson. 1999. Iron Overload in the Animal Kingdom. *In* Zoo and Wild Animal Medicine. 4th Ed. Fowler, M.E. and R.E. Miller, Eds. W.B. Saunders Co, Philadelphia, PA. p. 260 - 268.