ANNUAL REPORT OT THE POLLOCK CONSERVATION COOPERATIVE RESEARCH CENTER

Project title: "Thyroid Hormones and Plasma Leptin concentrations During Food Deprivation and Satiety: Use as an index of Metabolic Condition in Free- Ranging Steller Sea Lions"

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Summary

The objective of this study was to assess the use of a suite of physiological measurements for use in evaluating the body condition and overall health of Steller sea lions (SSL). The suite of measurements included the thyroid hormones (total and free T3 and T4), cortisol and leptin. Radioimmunoassays were validated for SSL serum and plasma. Most of the thyroid hormones (ie. tT4, tT3 and fT3) were highest in the first year of life and decreased to relatively level concentrations by Year 3 of life. Cortisol followed the same pattern. Leptin varied by season with concentrations highest in the winter and spring. When individual hormones were plotted against body mass. a significant increase in tT3 and leptin occurred around 100-150 kg body mass. Sea lions in this weigt class tend to be around 18 months of age. The increase in t T3 indicates an elevation in metabolic rate of these animals and could be due to a variety of factors (weaning, molt, inability to thermoregulate). We suggest that the physiology of juvenile SSL's needs to be studied to understand the impact of environmental change on metabolic homeostasis in young developing sea lions.

Background

In marine mammals, such as Steller sea lions. the physiological role of fat and the animals metabolic rate is slightly different than in other terrestrial mammals. Marine mammals are dependant on fat for thermoregulation against their relatively cold water environment, buoyancy, and energy storage. Many pinnipeds undergo seasonal fasting periods during summer breeding events. Female Steller sea lions may fast for one to two weeks while nursing a new pup. Males defending a territory may fast four to five months while maintaining extremely active energy expenditures. fighting to control territory and access to females. Also, newborn pups may fast for days at a time waiting for 'mom' to return from a foraging trip with sustenance. During any of these phases, animals will lose body fat. Unlike other mammals, this seasonal or developmental metabolism of blubber energy stores is a product of the evolution of this species to a semi-aquatic lifestyle.

Adipose tissue is known to maintain the largest energy reserves in the animal body. In addition to this role, recent research suggests that adipocytes may function as a center in the regulation of energy management. Adipose tissue is responsive to a number of endocrine signals (Hwang et al., 1997). A adipocyte-specific gene, the *obese* gene, was recently identified and found to encode a hormone that plays a major role in the regulation of energy intake and expenditure. Leptin, commonly referred to as the *ob* or obesity protein, is a peptide hormone secreted by adipocytes (Campfield et al., 1996). Leptin, and the recent elucidation of the fundamentals of its regulatory physiology, has been identified as a plausible candidate for a humoral signal with the requisite endocrinology and neurobiology that may act to integrate somatic energy stores, energy expenditure and fertility (Rosenbaum and Leibel, 1998). The specific molecular and biochemical pathways of action associated with leptin are still being intensively studied. However, it is thought that leptin acts as a negative feedback signal to satiety centers in the hypothalamus to regulate body energy stores. High levels of leptin, found in association with abundant adipose reserves, are secreted and signal the brain to regulate energy balance or in this case, decrease food intake.

Research on mice, lemmings, humans and non-human primates has shown that leptin is a hormone secreted from the adipose tissue that acts on the central nervous system centers to influence food intake and energy balance. The role of leptin in comparative animal systems has received much less attention to date, although significant advances have been made in the study of hibernating mammals. The gene that codes for the production of leptin is expressed only in adipose tissue and leptin is produced and secreted in proportion to the size and number of fat cells present (Rea and Nagy, 2000). In several species studied to date, it has been demonstrated that a close correlation exists between the blood leptin concentration and total body fat mass. If a relationship between leptin and body fat content could also be established for Steller sea lions, this hormone could provide an index of body condition that could be more easily monitored in free-ranging animals than the presently used bioimpedence or deuterium dilution technique.

Sample Collection and Methodology

Steller sea lion blood samples were collected by Alaska Department of Fish and Game (ADF&G) in collaboration with National Marine Fisheries Service (NMFS) during the summer field seasons of 2000 and 2001. Approximately 460 blood samples from juvenile animals were collected in the last two field seasons. In addition, several archived samples have been made available and are included in this study. All hormone assays were completed in Dr. Shannon Atkinson's laboratory at the ASLC. All blood samples, once in possession, were kept frozen at approximately -80°C until assayed.

Leptin concentrations were determined by radioimmunoassay (RIA) using the Multi-species Leptin RIA kit (Linco, St. Charles, MO) and have been validated for Steller sea lions. In this type of assay, ^{125}I-labeled hormone competes for a fixed time with hormone in the animal sample for antibody sites. After a specified time, the supernatant is decanted causing immobilization of the antibody, termination of competition and

isolation of the antibody-bound fraction of the radiolabeled hormone. A gamma counter yields a number that is converted to a measure via a calibration curve. A standard curve for each assay will be log-logit transformed, enabling extrapolation of sample concentration. Nonspecific binding and the sensitivity of assay used were considered. Nonspecific binding of other proteins from the sample is considered minimal. Sensitivity was indicated by the detection limit or minimal detectable dose defined as the apparent concentration at 95% maximum binding/nonspecific binding to specify that samples fell into the range. Again, parallelism and linearity tests indicate that the kits work for Steller sea lions. Validation of cross-reactivity with Steller sea lion serum will be confirmed through the analysis of dilution curves. Leptin concentration will be compared to existing data on morphometrics and body fat content values as determined by bioimpedence and D_2O (deuterium oxide) procedures.

Analysis of variance will be used to compare results of the individual tests among animals at different sampling sites. Repeated measures analyses of variance (ANOVA) and Tukey Kramer post hoc multiple comparison tests will be performed separately to consider relationships between hormone concentration and morphometric data ($p< 0.05$). A Student's t-test will be performed to test for significant difference between hormone concentrations. Linear regression analyses will be employed to test for significant correlation between serum leptin levels and the animals' total body fat (arc sin transformation to approximate a normal distribution of data) and other hormone levels being investigated (thyroid hormones and cortisol). Correlation analysis will be used to identify significant relations among the various analyses and chemical body burden. Multivariate techniques (*e.g.*, principal components analysis) will be used to summarize the variance in the different tests used.

Preliminary Data

The objective of this study was to develop and investigate the suitability of using a blood sample and the subsequent analysis of leptin hormone concentrations as a correlate to fitness in free-ranging Steller sea lions. Recent studies on terrestrial mammals have shown a close correlation between serum leptin levels and total body fat. If leptin measurements can provide an index of body condition in free-ranging Steller sea lions, it would be a useful screening technique to compare between sea lions across their geographic range. In addition, this study has built upon a previous preliminary study that looked at the effect of food deprivation on serum leptin concentrations. Free-ranging Steller sea lions (n=49) were compared to captive animals (n=3) and validation indicates our assay procedures work well for this species. There was no significant difference between serum and plasma or between males and females. Samples from wild Steller sea lion populations (with age ranging form four to 16 years) indicate ranges of leptin concentrations from 0.3 to 4.9 ng/ml with a mean of 2.1 ± 1.2 ng/ml. Interestingly, concentration ranges for captive animals (all age seven years) are narrower at 1.2 to 3.8 ng/ml with a mean of 2.6 ± 0.6 ng/ml. The preliminary results indicate that leptin may be a useful indicator of body size and condition.

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