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CHANGES IN SERUM LEPTIN LEVELS DURING FASTING AND FOOD LIMITATION IN STELLER SEA LIONS *(EUMETOPIAS JUBATUS).*

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Introduction

Leptin, also commonly known as the *ob* protein, is a peptide hormone secreted by adipocytes which has been shown to have a role in energy metabolism and food intake in rodents and man (Campfield et al. 1996); Although the specific molecular and biochemical pathways of action of this hormoneare still the-focus of intensive study, it is thought that leptin acts as a negative feedback signal to satiety centers in the hypothalmus to regulate body energy stores. When adipose reserves are abundant, high levels of leptin are secreted and signal the brain to regulate energy balance (i.e. decrease food intake). The role of leptin in other animal systems has received much less attention to date, thus we chose to investigate how serum leptin concentrations change in response to food deprivation in an animal which is known to undergo periods of voluntary natural fasting in the wild. Female Steller sea lions fast for 1 to 2 weeks during the summer breeding season in order to give birth and nurse their young. Males are also thought to fast while defending territory during the breeding season. By simulating these fasting bouts in a captive environment the effect of complete fasting and body condition (i.e. total fat content) on circulating leptin levels could be addressed.

In rodents and humans, food intake has been shown to increase leptin production and fasting consistently decreased leptin secretion by the adipocytes (Saladin et al. 1995, Pratley et al. 1997). To address the related, but individual effects of fasting and decrease in body reserves on leptin production we also held sea lions on a low plane of nutrition (food limitation) for 28 d such that body mass loss was similar to that experienced during 14 d fasting experiments.

In several species studied to date, a close correlation between serum leptin concentrations and total body fat mass has been demonstrated. If a close correlation 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. Presently the best method for determining body fat content in these animals involves holding captured individuals under an aesthesia during the two hour equilibration period necessary for the dilution of deuterium.

Methods

Blood samples were collected during experimental food limitation and complete fasting trials conducted on subadult Steller sea lions held in captivity at the Vancouver Aquarium, Vancouver, British Columbia, Canada, During food limitation studies 4 animals (3M:1F) were fed previously frozen herring *(Clupea* spp.) ate rate below normal intake that resulted in a 0.5 kg d^{-1} mean rate of body mass loss. Blood samples were collected on the first d of study after an overnight fast (0830 to 1030h, approximately 16 hours after last meal) and then every 7 d during 28 d of food restriction (each after overnight fasts). Blood chemistry and physical characteristics of the serum suggested that these animals were in a post-absorptive state. Five subadult sea lions (3M:2F) also underwent complete fasting trials during which blood samples were collected after an overnight fast and then every 3 to 4 d up to a maximum of 14 d of fasting or 15% body mass loss, whichever occurred first. In addition, blood samples were collected from 4 young Steller sea lion pups (approximately 2 months of age) after an overnight fast and after 1.5 and 2.5 d of fasting. Blood samples were centrifuged immediately and remained frozen until analysis at University of Alabama at Birmingham.

Leptin concentrations were determined by radioimmunoassay (RIA) using the Multi-species Leptin RIA kit (Linco, St. Charles, MO, kit # XL 85K) on 100 ul samples of serum. Validation of cross-reactivity with Steller sea lion serum was confirmed through the analysis of dilution curves. Plasma water content was determined by dessication of 400 ul of plasma in a drying oven at 80°C for 72 hours, calculated on a mass basis and corrected for specific gravity of the plasma to determine percent water volume.

Body fat content was determined by the dilution of deuterium oxide after an overnight fast at the beginning of each study, and on the final day of blood collections for each experiment. Pre-injection blood samples were compared to blood samples collected 2 to 2.5 hours after injection of approximately 10 ml deuterium oxide (0.1 to 0.5 g kg^{-1} body mass, 99.9% enriched, Isotec, Miamisburg, Ohio).

Students t-tests were used to determine significant difference between leptin concentration change in males and in females during either food limitation or complete fasting treatments (a < 0.05). Repeated measures analyses of variance and Tukey Kramer post hoc multiple comparison tests were performed separately for males and females to determine significant changes in serum leptin concentrationsduring the complete fasting and food limitation trials (a < 0.05). A Student's t-test was performed to test for significant difference between leptin concentration on the first day of study (after overnight fast) in subadult animals versus four young pups (approximately 2 months of age, fasted for 3 d). Linear regression analyses were employed to test for significant correlation between serum leptin levels and the animals total body fat or plasma water content (arc sin transformation to approximate a normal distribution of data).

Results

Males and females showed significantly different patterns of change in serum leptin concentration during fasting experiments (n=5; p=0.031) (Figure 1). The-three male sea lions showed a consistent decrease in serum leptin levels during the 9 to 14 d fasting period, while the two female sea lions shown progressive increase in serum leptin concentrations over the same period of fasting. Serum leptin patterns were less distinct during food limitation studies, with two animals (both male) showing progressive decrease of leptin with food restriction, while the remaining two animals (one male, one female) increased leptin concentrations within the first 2 weeks of the limitation study followed by subsequent progressive decreases (Figure 1). Mean serum leptin concentration on the first day of study (overnight fast) in subadult animals (6.41 ± 1.13 ng ml^{-1}; n=9) was significantly higher than circulating serum leptin levels in young pups after an overnight fast (3.47 ± 0.31 ng ml^{-1}; n=4).

Due to the small sample sizes available when analyzing males and females separately, changes seen during fasting and limitation trials within individuals were not statisticajly significant (p values range from 0.157 to 0.477). There was also no significant difference between magnitude of changes in leptin concentration seen in individuals during fasting versus limitation trials when compared either during the first 7 d of study (paired t-test, n=4; p=0.212) or during the period required to reach 12 to 15% body mass loss (paired t-test, n=4; p=0.317). No significant changes in leptin concentration were evident during 2.5 d of fasting in young Steller sea lion pups (n=4; p=0.762).

Serum leptin concentrations were not significantly correlated with total body fat mass as' measured by the dilution of deuterium oxide (p=0.627; Figure 2) but were significantly correlated with plasma water content ($p<0.001$, $R^2=0.49$; Figure 3).

Discussion

Several aspects of this preliminary data set have presented unexpected results. Male sea lions showed decreases in leptin concentration (although not statistically significantfor this sample size) as is seen during fasting in rodents and humans (Saladin et al 1995, Pratley et al. 1997). However, Steller sea lion females showed opposite trends in serum leptin concentrations during fasting. Previous studies on fasting humans and rats have shown no evidence of this sex based difference in response to fasting. 'The close relationship between serum leptin concentrations and total body fat content that has been shown in several other mammals studied was not apparent in the 11 samples collected after overnight fasting in this study. Animals with greater body fat mass did not circulate higher levels of leptin as would be expected if leptin was produced. strictly in proportion to the number and volume of adipocytes. There are clearly other factors influencing serum leptin concentration, and there is evidence that the distribution of these lipid reserves may play a critical role in determining the expression of the *ob* gene (Nagy et al. 1997). We plan to investigate these relationships further through analysis of blood samples available from 10 additional fasting experiments and through analysis of concurrent changes in blubber depth measured during fasting trials using a ScanProbe portable ultrasound unit.

At present, we conclude that young animals produce significantly lower levels of leptin than do older, larger sea lions, however it is unclear the role that body fat content plays in this difference in leptin concentration. There are also clearly differences in how serum leptin levels change in response to fasting in males and females of this species, although the factors influencing leptin production in Steller sea lions require further study.

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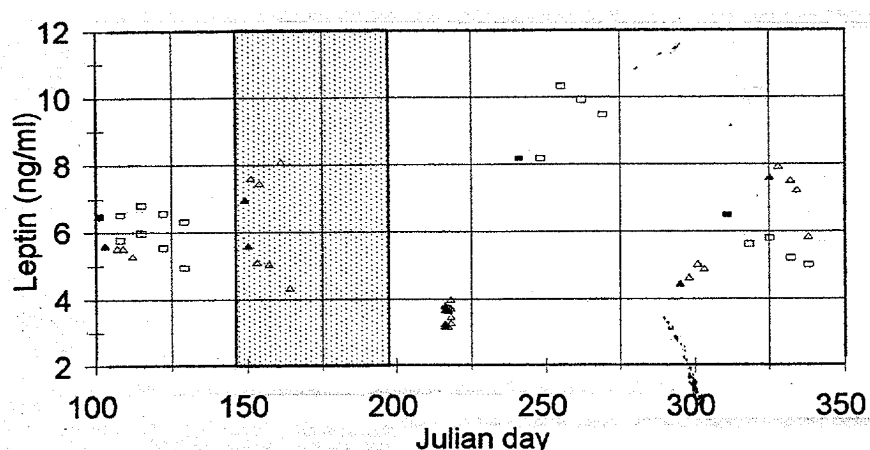

Figure 1. Serum leptin concentrations (ng/ml) during fasting (▲,△) and food limitation (□, □) experiments in captive Steller sea lions.

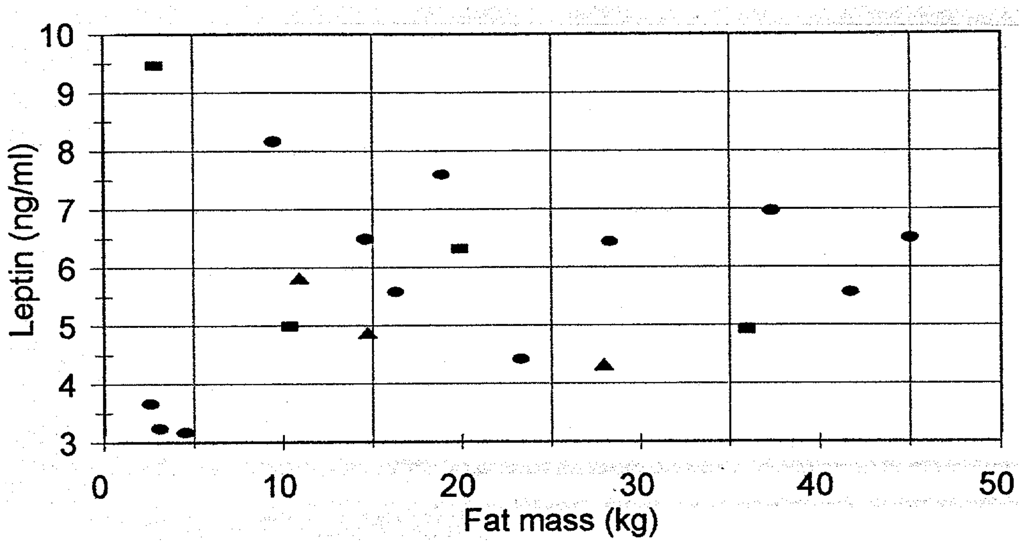

Figure 2. Relationship between serum leptin concentration (ng/ml) and total body fat mass (kg) in captive Steller sea lions. • represent animals sampled after an overnight fast, ▲ represent animals fasted 14 d, and ❑ represent 28 d food limitation trials..

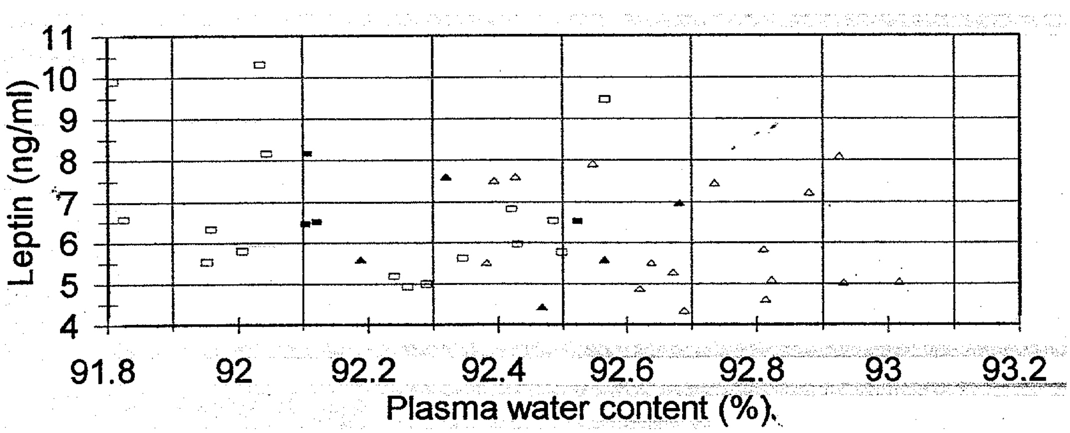

Figure 3. Relationship between serum leptin concentration (ng ml) and plasma water content (%vol/vol) in Steller sea lions during fasting (▲, △) and food limitation (□, □) experiments.