

Effect of Prey Composition on the Endocrine Response to Nutrient Restriction and Re-alimentation in Steller sea lions (*Eumetopias jubatus*)

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Introduction

The dramatic decline of Steller sea lion populations (approximately 80%) over the last 30 years has brought about significant research focus to determine the cause of the decline. While many plausible hypotheses have been put forth, nutritional stress has not been eliminated. However, little is known about the mechanism in which decreased nutrient intake influences the physiology of Steller sea lions. By investigating the factors that link nutrition, fat metabolism and lean tissue accretion, we can assess the impact of decreased nutrient intake on energy storage and lean tissue growth, which may have implications for survival.

The metabolic hormones, growth hormone (GH) and insulin-like growth factor (IGF)-I, together with the IGF binding proteins (BP), are the primary components of the somatotropic axis, which is associated with growth rate and accretion of protein and adipose (Lawrence and Fowler, 1997). There are six IGFBP, which regulate the biological actions of IGF-I (Jones and Clemmons, 1995), but IGFBP-2 and -3 are most associated with growth rate (Govoni et al., 2003). In many species, measures of the somatotropic axis are predictive of nutritional status and future growth rate (Freake et al., 2001; Rausch et al., 2002). Research on the somatotropic axis has mainly focused on domestic species with little information available for wildlife.

Nutritional status of an individual animal influences the somatotropic axis in predictable ways. Compared with animals fed ad libitum, animals fed a restricted diet over an extended period of time have reduced growth rate, coupled with reduced concentrations of IGF-I and IGFBP-3 and increased concentrations of GH and IGFBP-2 (Rausch et al., 2002). This nutrition-induced increase in GH is due to decreased metabolic clearance rate of GH (Breier, 1999). The primary role of GH is to preserve body protein, especially in times of energy deficit (Johnsson and Hart, 1986). In contrast, IGF-I concentrations are decreased with inadequate nutrition, which is due in part to the down regulation of hepatic GH receptors, where the majority of circulating IGF-I is produced (Strauss, 1994), leading to hepatic GH resistance. Similar to IGF-I, concentrations of IGFBP-3 are reduced with nutrient deficit and may also be due to decreased GH sensitivity in the liver (Smith et al., 1995). However, during limited nutrient availability, IGFBP -2 is increased, reducing availability of IGF-I for peripheral tissues (Renaville et al., 2000).

Composition of the diet can also influence components of the somatotrophic axis. For example, limitation of dietary protein is associated with reduced growth rate, IGF-I and IGFBP-3, but increased IGFBP-2 (Kriegl et al., 1992). Thus, changes in the quality and (or) quantity of feed can influence components of the somatotrophic axis, resulting in variation in the changes observed with age and (or) sex. This experiment was performed to investigate the effects of reduced nutrient intake and diet composition on components of the somatotrophic axis in Steller sea lions.

Methods

Animals and Diets

Captive Steller sea lion females ($n = 8$, 2 to 5 year of age) were used to examine changes in the somatotrophic axis in response to decreased nutrient intake. Animals were placed on a normal herring maintenance diet for 1 month. After this 1 month 'baseline' period four animals were placed on a herring submaintenance diet and four animals were fed an isocaloric Pollock submaintenance diet for 1 month. Pollock has a lower energy density, lower lipid content, and greater water content compared with herring. Previous research has shown that Steller sea lions fed submaintenance isocaloric diets lose more lipid mass when they are fed fish with a lower fat content (Rosen and Trites, 2005). This illustrates the importance of diet composition on energy maintenance. Following this 1 month period of sub-maintenance feeding, animals were placed back on the original normal herring diet for an additional 1 month re-alimentation period. Blood collection, morphometrics, body composition (determined by deuterium dilution) and blubber depth (assessed by ultrasound) were performed starting at the initiation of the submaintenance diet and repeated every 2 week until the end of the experiment.

Blood handling and assays for ST, IGF-I and IGF binding proteins

Sera was analyzed by radioimmunoassay for GH (Kazmer et al., 1992) and IGF-I (Rausch et al., 2002). We have validated the assay for quantification of IGF-I and GH in Steller sea lions. Serially diluted pooled serum from Steller sea lions was parallel to the standard curve for both IGF-I ($R^2 = 0.993$) and GH ($R^2 = 0.986$), and percent recovery of added mass was 95 to 101% and 90 TO 107 % for IGF-I and GH, respectively.

Concentrations of IGFBP -2 and -3 were determined by ligand blot following polyacrylamide gel electrophoresis (Freake et al., 2001). Following transfer to a nitrocellulose membrane, concentrations of IGFBP were quantified by determining the amount of labeled IGF bound to the sample and expressed as a percentage of the quantity of IGF bound to IGFBP standard on the blot.

Statistical analyses.

Longitudinal measurements of serum ST, IGF-I, and IGFBP data were analyzed as repeated measures in a subplot, using a model that accounts for repeated samples from the same experimental unit (Gill and Hafis, 1971), with the mixed model analysis of variance procedure of SAS (SAS Inst. Inc., Cary, NC). Correlations of ST, IGF-I, IGFBP-2 and -3 and body weight used Spearman's correlation procedures in SAS (1989). Differences were considered significant if $P \leq 0.05$.

Results and Discussion

Somatotropic Axis Components

During the 1 month submaintenance period, the animals lost 10 to 15% of their body mass. In the 1 month re-alimentation period, only three animals increased mass to their initial value. The other five animals remained 2 to 10% less than their starting mass. All other individuals gained approximately 1% of body mass between week 9 and 11 of the re-alimentation period. There was no apparent effect of diet on mass or somatotropic axis components measured ($P > 0.05$).

Concentrations of IGF-I followed the expected pattern paralleling changes in intake. Mass and IGF-I declined when animals were fed a submaintenance diet and began to increase once nutrient intake was increased to maintenance requirements (Figure 1). However, even after 3 weeks of recovery diet, IGF-I concentrations were still less than initial concentrations (162 ng/ml vs. 316 ng/ml). An additional sample was taken from three individuals after 8 weeks of the recovery diet and demonstrated a further increase in IGF-I (226 ng/ml). This increase was slower than anticipated and may indicate that the maintenance diet was not sufficient to meet energy requirements after an extended submaintenance diet. Little change was observed in IGFBP-2 throughout the experiment.

Concentrations of GH were more variable than IGF-I (Figure 2). Serum samples were taken at the same time of day and relative to the last meal consumed, however GH concentrations were more variable than IGF-I due to the pulsatile nature of GH secretion. The pattern of change in GH was unexpected based on research in domestic species. Concentrations of GH generally increase with decreased nutrient intake (Rausch et al., 2002). In this experiment there was a slight increase in GH when animals were fed the submaintenance diet; however, a greater peak in GH was observed after animals were placed on the recovery diet. This peak after animals had been on the re-alimentation diet for 3 weeks may indicate an increase in lean tissue accretion (Breier, 1999). Research in domestic species has shown that GH may continue to be greater in the recovery phase (compared with controls) after food restriction (Hornick et al., 2000). This failure of GH to decline once intake was increased may be further evidence that nutrition was not adequate to meet maintenance requirements after nutritional limitation.

Concentrations of IGFBP generally followed the expected response based on domestic animal research. The overall concentration of IGFBP-3 declined with decreased nutrient intake. In contrast, IGFBP-2 increased with decreasing nutrient intake (Figure 3).

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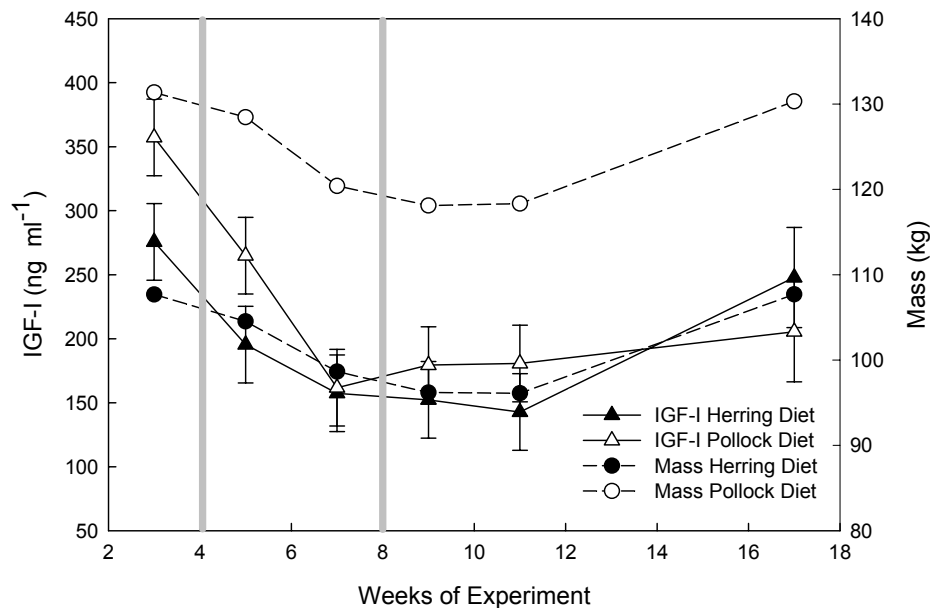


Figure 1. Concentration of IGF-I in Steller sea lion females fed two isocaloric submaintenance diets followed a pattern similar to mass. The herring diet was high in lipid while pollock had comparatively less lipid. Area between the gray bars is the submaintenance phase of the experiment. Values shown are mean \pm SE for IGF-I mass SE=14 kg.

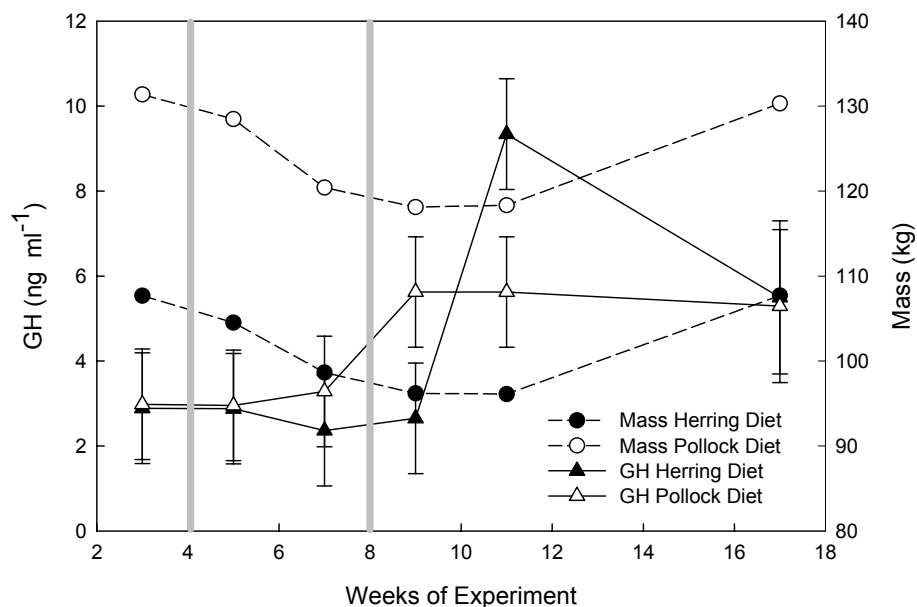


Figure 2. The pattern of change in GH followed a contrasting trend to the change in mass throughout the experiment. Area between the gray bars is the submaintenance phase of the experiment. Values shown are mean \pm SE for GH, mass SE=14 kg.

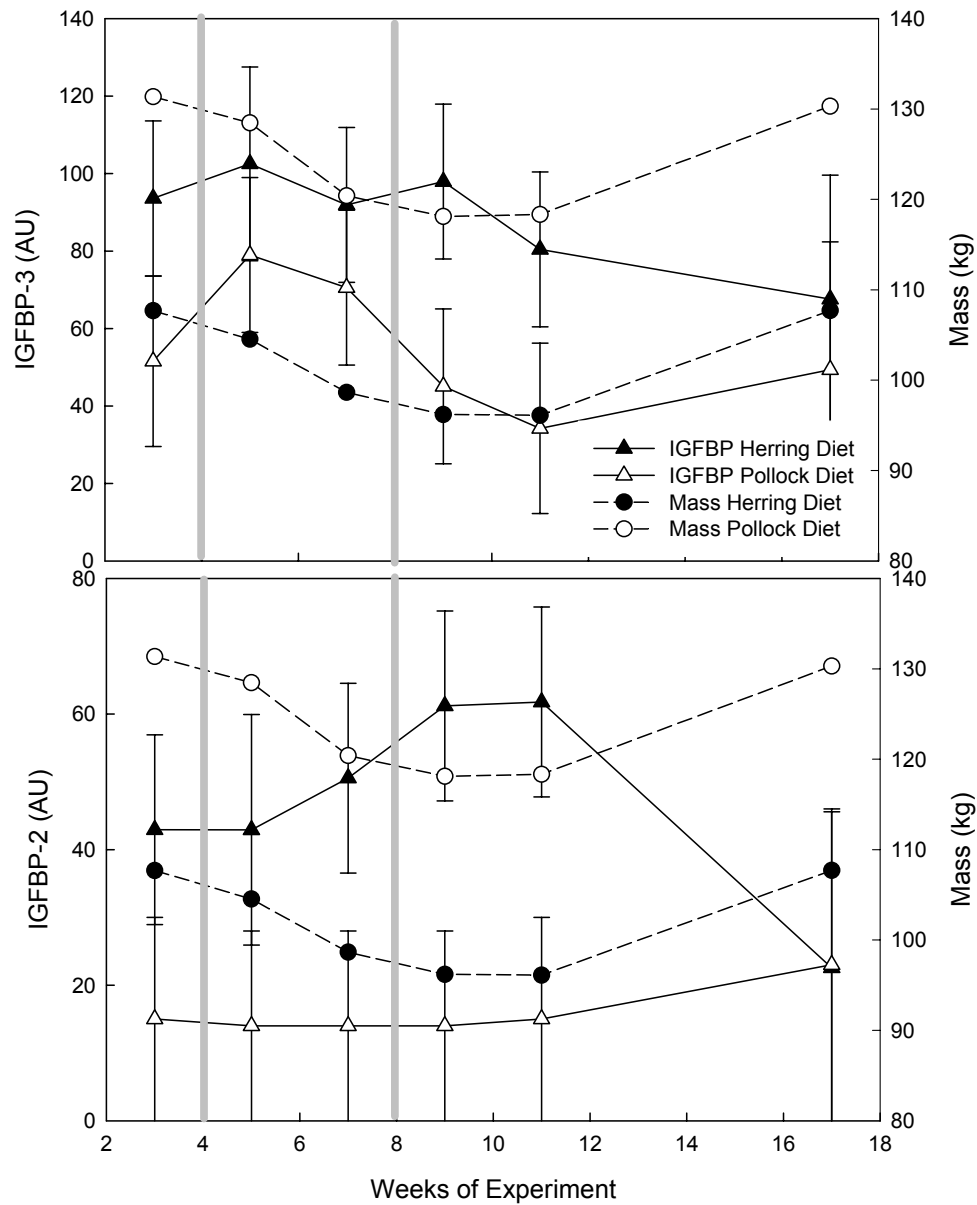


Figure 3. Concentration of IGFBP expressed in arbitrary units (AU). Concentration of IGFBP-3 followed a similar trend with the change mass, while the IGFBP-2 trend was opposite. Values shown are mean \pm SE, mass SE=14 kg.