General and Comparative Endocrinology 165 (2010) 286–292

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Re-alimentation in harbor seal pups: Effects on the somatotropic axis and growth rate

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ARTICLE INFO

Article history:
Received 18 March 2009
Revised 30 June 2009
Accepted 9 July 2009
Available online 14 July 2009

Keywords:
Condition index
Growth hormone
Growth rate
Harbor seal
Insulin-like growth factor-I
Insulin-like growth factor binding proteins
Phoca vitulina
Somatotropic axis

ABSTRACT

The metabolic hormones, growth hormone (GH) and insulin-like growth factor (IGF)-I, together with IGF binding proteins (IGFBP), have been well studied in domestic species and are the primary components of the somatotropic axis. This hormone axis is responsive to nutrient intake, associated with growth rate, and accretion of protein and adipose. However, this relationship has not been evaluated in species that rely heavily on adipose stores for survival, such as pinnipeds. The primary objectives of this research were to investigate the response of the somatotropic axis to reduced nutrient intake and re-alimentation in rehabilitated harbor seal pups, and to assess if these hormones are related to nutritional status and growth rate in harbor seals. Stranded harbor seal pups ($n = 24$) arrived at the rehabilitation facility very thin after fasting for several days (nutritional nadir). Throughout rehabilitation nutrient intake increased and pups gained mass and body condition. Concentrations of GH and IGFBP-2 decreased with re-alimentation, while IGF-I and IGFBP-3 concentrations increased. Overall, GH and IGFBP-2 were negatively associated and IGF-I and IGFBP-3 were positively associated with growth rate and increased body condition of harbor sea pups. Further, the magnitude of the growth response was related to the magnitude in response of the somatotropic axis to varied levels of intake. These data suggest that multiple components of the somatotropic axis may be used to assess the energy status of individuals and may also provide information on the level of feed intake that is predictive of growth rate.

1. Introduction

The metabolic hormones, growth hormone (GH) and insulin-like growth factor (IGF)-I, together with the IGF binding proteins (IGFBP), 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 and are responsive to nutrient intake (Sohlström et al., 1998; Rausch et al., 2002; Govoni et al., 2003). Research on the somatotropic axis has primarily focused on domestic species with little information available for wildlife.

Research on domestic animals and humans has shown that 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 (Straus, 1994; Breier 1999; Harrell et al., 1999; Rausch et al., 2002). This increase in circulating GH concentration stimulated by reduced nutrient intake is due to decreased clearance rate of GH (Breier, 1999). In many species, measures of the somatotropic axis are predictive of nutritional status and future growth rate (Connor et al., 2000; Renaville et al., 2000; Freake et al., 2001; Rausch et al., 2002).

The limited information that exists for components of the somatotropic axis in marine mammals suggests that GH and IGF-I in phocids respond to fasting and increased intake in a similar manner compared with other mammalian species (Ortiz et al., 2003; Eisert et al., 2005; Richmond et al., 2008). Concentrations of GH increased and IGF-I concentrations decrease in fasting northern elephant seals (Ortiz et al., 2003), and increased nutrient intake stimulates an increase in IGF-I concentrations in adult female Weddell seals (Eisert et al., 2005). However, detailed investigation of the response of multiple components of the somatotropic axis to changes in nutritional state in young pinnipeds has not been conducted.

Harbor seals (*Phoca vitulina*) are annual breeders, and most pups in California are born within a 2-week period in late March to early April (Burns, 2002). Pups average 8–12 kg at birth and typically nurse for 4–6 week on high fat milk (50% lipid), and gain an

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doi:10.1016/j.ygcen.2009.07.007

average of 0.6–0.7 kg d^{-1} throughout nursing, which corresponds to 5–9% daily increases in body mass (Bowen et al., 2001a; Schulz and Bowen, 2004; Lang et al., 2005). This rapid increase in overall body mass includes rapid deposition of lipid (blubber) which is essential to their survival (Muelbert et al., 2003; Burns et al., 2005). Indeed, the first year survival of harbor seal pups is positively correlated with body mass and body lipid, with larger pups having a greater survival rate (Muelbert et al., 2003; Harding et al., 2005). Therefore, pups with a greater growth rate leading to increased size may also have a greater rate of survival.

The primary objectives of this research were to investigate the response of the somatotropic axis to reduced nutrient intake and re-alimentation in harbor seal pups, and to assess if these hormones are related to nutritional status and growth rate in harbor seals. Rehabilitation of healthy but abandoned harbor seal pups provide a unique model to study the physiologic effects of reduced nutrient intake and re-alimentation because they arrive at rehabilitation at a low nutritional state after fasting for several days (Richmond et al., 2008). During the subsequent rehabilitation process, animals are fed and proceed through the re-alimentation process gaining body mass and adipose stores (Richmond et al., 2008). This experimental model provides an opportunity to conduct a longitudinal experiment on pinnipeds from natural genetic stock with known nutritional status and intake. We hypothesize that the somatotropic axis in harbor seals will respond to reduced nutrition and re-alimentation as predicted by the domestic animal model.

2. Methods

2.1. Animals and diet

Abandoned harbor seal (*Phoca vitulina richardsii*) pups brought to The Marine Mammal Center (TMMC, Sausalito CA) were initially assessed by a veterinarian and determined to be healthy but abandoned through physical examination, body weight measurement, hematology and serum chemistry panel. Only pups that met this "healthy but abandoned" status were used for this study. Age was estimated through a combination of umbilicus condition, percentage of lanugo fur and tooth eruption (Boulva, 1975; Bowen et al., 1994; Gulland et al., 1997). At rescue, seals had typically been fasting from one to several days (nutritional nadir) as determined by observation of more than 24 h without maternal care before rescue and thin appearance for age.

Pups were fed a Multi-Milk formula (Pet-Ag, Inc., Hampshire, IL) with fish oil, lecithin granules, and a pinniped multi-vitamin (Mazuri, Purina Mills, Inc., St. Louis, MO) five times a day for approximately 4 week and were then weaned onto a mixed fish diet consisting primarily of North Atlantic and Pacific herring (*Clupea* spp.) (Gage, 2002; Lander et al., 2003). Intake was recorded daily. Formula contained approximately 36.0% fat, 6.1%, protein, 46% water and 3700 cal L^{-1}. The energy content of the herring (1500 cal kg^{-1}) was estimated by proximate composition analysis of batches of fresh frozen herring of similar age class performed by Mystic Aquarium & Institute for Exploration.

The study was conducted over two years (2005 and 2006). Fourteen pups each year (11 F, 3 M year 1; 8 F, 6 M year 2) met the criteria for healthy but abandoned. Based on the aging criteria, all pups in this study were less than 10 days of age and most were estimated to be approximately 5 days of age at initial assessment. Mass was measured every week and blood samples (2 ml) were collected from each pup every 2 weeks for approximately 11 week or until release. Growth rate was calculated by determining the change from initial body mass divided by the days in rehabilitation and was reported in kg d^{-1}. In the first year of the study (2005) standard length was assessed at the time of arrival and again at release. In the second year (2006), standard length and axillary girth were measured every two weeks. A condition index (axillary girth/standard length × 100) was calculated for pups in 2006 (Fadely, 1997).

2.2. Blood handling and assays for GH, IGF-I and IGF binding proteins

Before the first feeding of the day, after an 8 h overnight fast, animals were manually restrained and blood was collected via venipuncture of the extradural intravertebral vein (Bossart et al., 2001). Blood was allowed to clot for 15 min and then centrifuged at 3000*g* for 15 min at 4 °C. Sera were frozen and shipped on dry ice to the University of Connecticut for analysis. Samples were maintained at −80 °C until processing.

Sera were analyzed by heterologous radioimmunoassays validated for quantification of IGF-I and GH in harbor seals (Richmond and Zinn, 2009). Serially diluted pooled serum 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 90–99% and 90–107% for IGF-I and GH, respectively. Growth hormone intra- and inter-assay coefficient of variation (CV) were less than 6% and 9%, respectively, for low (1.3 ng ml^{-1}) and high (4.4 ng ml^{-1}) GH pools. The IGF-I intra- and inter-assay CV were 5% and 8% for low (68 ng ml^{-1}) and 7% and 15% for high (267 ng ml^{-1}) IGF-I pools, respectively.

Among the six IGFBP currently identified, IGFBP-2 and -3 are useful indicators of growth rate and nutritional status (Straus and Takemoto, 1990; Smith et al., 1995; Govoni et al., 2003) and therefore the focus of our investigations. Concentrations of IGFBP-2 and -3 were determined by ligand blot following polyacrylamide gel electrophoresis (Freake et al., 2001). Membranes were incubated overnight with approximately 1.6 MBq of ^{125}I-labeled IGF-I (GE Healthcare Bio-Sciences Corporation, Piscataway, NJ). After incubation, membranes were washed to remove unbound ^{125}I-labeled IGF-I and then exposed to a multipurpose phosphor screen (Packard Instrument Company, Meriden, CT). The remaining radioactivity bound to blots was imaged with a Cyclone Storage Phosphor System (Packard Instrument Company), and quantified with OptiQuant software (Packard Instrument Company). Binding proteins were quantified as digital light units per mm^2 and expressed in arbitrary units (AU) as a percentage of the pooled serum standard IGFBP-3 included on each gel. Intra- and inter-assay CV were less than 4% and 7%, respectively, for both IGFBP-2 and -3. Serially diluted pooled serum exhibited dilution linearity for both IGBP-2 (R^2 = 0.998) and IGFBP-3 (R^2 = 0.983).

2.3. Statistical analyses

Longitudinal measurements of serum hormone concentrations and morphometrics were analyzed as repeated measures with the mixed model analysis of variance procedure (SAS Inst. Inc., Cary, NC). This model accounts for repeated samples from the same experimental unit (Gill and Hafs, 1971). The statistical model initially included sex, year, and week of rehabilitation as independent variables; however, gender did not significantly affect any variable, except mass, growth rate, intake, and IGFBP-2 concentration, therefore gender was removed from the model for all other variables. The reduced statistical model increases the power to detect differences. The final model included week of rehabilitation, year, and the interaction of week × year. Because axillary girth was not measured in 2005, year was not included in the model for axillary girth or condition index. To evaluate differences among weeks of rehabilitation, a pair wise comparison of the Least Significant Difference was used. Differences were considered significant if $P \leqslant 0.05$ and trends if $0.10 > P > 0.05$. A stepwise linear regression analysis was conducted to assess the relationship between