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Endocrine response to realimentation in young northern elephant seals (*Mirounga angustirostris*): Indications for development of fasting adaptation

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ABSTRACT

Most organisms undergo changes in their environment, both predictably and unpredictably, which require them to alter priorities in nutrient allocation with regards to food availability. Species that more predictably encounter extended periods of limited food resources or intake while mitigating the negative effects of starvation are considered to be fasting adapted. Northern elephant seals (NES) are one such species and routinely undergo extended periods of fasting for breeding, molting, as well as a post-weaning fast at 6–8 weeks of age. However, during unusual times of nutritional deprivation, animals may enter stage III fasting. While fasting and foraging in this species has been extensively studied, realimentation following fasting beyond normal life history parameters has not been investigated. In this study, changes in ghrelin, growth hormone (GH), and insulin-like growth factor (IGF)-I were compared across 8 weeks of realimentation following emaciation in three age classes: neonates, post-molt pups, and yearlings. Longitudinal changes in hormone profiles indicate that neonate and post-molt pups are slow to recover mass and positive energy balance despite an energy dense diet fed at 10% body mass. In addition, ghrelin and GH concentrations remained elevated in post-molt pups compared to other age classes. Changes in hormone concentrations early in realimentation indicate that yearling animals recover more rapidly from periods of nutritional deprivation than do younger animals. Overall, this suggests that the ability to regulate metabolic homeostasis with regards to nutrient allocation may develop over time, even in a species that is considered to be fasting adapted.

1. Introduction

Throughout life, many organisms experience alterations in the need and ability to respond to physiological stressors. This may include altering patterns of nutrient utilization and allocation to cope with changes in tissues specific requirements such as reproduction, molting, seasonal adaptation, and periods of reduced nutrient intake. The somatotropic axis is a group of hormones that participates in the regulation of nutrient utilization and allocation (Breier, 1999; Etherton, 2004). Components of this axis [growth hormone (GH) and insulin-like growth factor (IGF)-I] are responsive to the nutritional status and physiological state of an individual (Breier, 1999; Thissen et al., 1999). In addition, the satiety hormone ghrelin contributes to the partitioning of nutrients through the promotion of feeding and adiposity (Sangiao-Alvarellos et al., 2011; Tschöp et al., 2000).

Models developed in domestic species have primarily defined the actions of these hormones with respect to changes in concentration and cellular action during changes in nutrient intake as well as with age. When nutrient intake is decreased, such as during a fast, IGF-I concentrations decrease while GH increases (Breier, 1999). Decreasing concentrations of IGF-I halt the processes of somatic growth when nutrient intake is low (Thissen et al., 1999). Increases in GH facilitate lipolysis to mobilize fatty acids from adipose as an energy source (Wang et al., 2004). Ghrelin normally exhibits pre-prandial increases as well as increases during fasting (Cummings et al., 2002). Upon realimentation, the anabolic effects of the somatotropic axis are restored, facilitating the deposition of fat as well as the growth of lean tissue (Thissen et al., 1999). Ghrelin participates in weight recuperation during realimentation by promoting adiposity and facilitating lipogenesis (Sangiao-Alvarellos et al., 2011; Tschöp et al., 2000). While ghrelin

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is not known to change with age, the somatotropic axis contributes to the regulation of age related nutrient priorities, with decreases in GH and increases in IGF-I with age (Skaar et al., 1994).

In addition to the domestic animal model, GH, IGF-I and ghrelin have been studied in species considered to be fasting adapted. These species require mechanisms to maintain metabolic homeostasis to accomplish this extension of the fasting period without negative effects on health and survival. Fasting adaptation requires the ability to transition from the first (primary carbohydrate utilization) and extend the second phase (primary lipid utilization) of fasting, postponing protein utilization and organ damage for extended periods of time (Castellini and Rea, 1992; McCue, 2010). Northern elephant seals (NES) are one such species, routinely experiencing extended periods of fasting as a part of their normal life-history patterns. Unlike some other fasting adapted species, NES couple periods of fasting with energetically demanding activities such as mating (including defense of territory), molting, and lactation (Castellini and Rea, 1992; Champagne et al., 2012; Riedman, 1991). Given this unique fasting adaptation, significant research investigating fasting physiology has been conducted in this species. However, the endocrine response to realimentation (re-feeding following fasting) has not been investigated nor the impact of developmental stage on this response.

NES pups are born at approximately 40 kg (Kretzmann et al., 1993; Schulz and Bowen, 2005) and gain mass rapidly during the nursing period. Upon weaning at approximately 4 weeks old, pups weigh approximately 130 kg with 50% body fat (Kretzmann et al., 1993; Schulz and Bowen, 2005). The first natural extended fasting period for a NES pup is the post-weaning fast, which begins at approximately the same time as the first molt and continues for 6–8 weeks (Bowen, 1991; Kretzmann et al., 1993).

However, some pups are prematurely abandoned by their mothers and often experience a loss of homeostatic control over the fasting response, become emaciated, and have lower survival through the post-weaning period (Rea and Costa, 1992; Houser and Costa, 2003). Additionally, animals experience varying degrees of foraging success after the post-weaning fast through the first year (Le Boeuf et al., 1994). These animals are often the subject of human intervention and taken into rehabilitation facilities for controlled realimentation. This subset of animals, which experience extreme fasting beyond normal life history patterns, provide a unique opportunity to investigate the physiological response to realimentation following late stage fasting or whether the ability to recover may be influenced by developmental stage. Therefore, the objective of this study was to investigate the ability to regain homeostatic regulation of nutrient utilization and allocation via the response of ghrelin, GH, and IGF-I from nutritional nadir through realimentation (refeeding) in three age classes representing different developmental stages: neonates (less than 4 weeks of age), post-molt pups (>4–8 weeks of age), and yearlings (approximately 1 year of age).

2. Methods

2.1. Animals and diet

All animals used in this study had undergone routine veterinary care for malnutrition at The Marine Mammal Center (TMMC; Sausalito, CA) between 1996 and 2013. Animals were categorized upon admittance into one of three age classifications: neonate pups (<4 weeks of age; n = 6), post-molt pups (4–8 weeks of age; n = 18), and yearling (approximately 1 one year of age; n = 15). Age classifications were based on pelage (status of molt), umbilical cord status, tooth eruption, size, and time of year in relation to peak (or mean) pupping date (Le Boeuf et al., 1994).

Animals were included in the study if they had greater than three blood sampling events during the rehabilitation period. The TMMC veterinary staff determined time of release depending on overall health condition and body mass of the animals. Only blood samples taken at or before 8 weeks were included in the analysis based on the average length of rehabilitation at 8.9 weeks. Thus, some animals included in the study were in rehabilitation longer than the 8 weeks of the study period.

Animals classified as neonate pups (younger than weaning age) were initially fed milk matrix formula (Zoologic 30/55, Pet-Ag, Inc. Hampshire, IL; 31.3% protein, 55.8% fat, and 3.1% carbohydrate) diluted with water and supplemented with fish oil and pinniped multivitamins. Formula was approximately 2100 kcal/L and amount fed was prescribed by veterinary staff at TMMC based upon estimated caloric requirement for growth at three times the resting energy requirements (RER; kcal/day) = 70 * (Body weight in kg)$^{0.75}$ (Lavigne et al., 1986). Pups were fed via gastric intubation approximately 5 times per day for 4 weeks (Townsend and Gage, 2001). At approximately 4 weeks of age, pups were gradually weaned from formula to whole herring (1500 kcal/kg), fed at 10% of body weight per day (Richmond et al., 2010). Animals older than 4 weeks (post-molt and yearling age classifications) were initially fed a fish mash (900 kcal/L) diet via gavage based on RER until transitioning to whole herring (10% of body mass) when able to eat on their own. Approximate daily caloric intake (kcal) was calculated based upon recorded actual intake of prescribed diet (mL and/or kg) and caloric content of formula (3700 kcal L^{-1}) and fish (1500 kcal kg^{-1}) (Townsend and Gage, 2001; Richmond et al., 2010).

Mass was measured to the nearest 0.5 kg, at minimum, at admit and release, with most animals weighed at each blood collection (approximately every 2 weeks). Standard length (SL) was measured to the nearest centimeter at admit and prior to release to assess growth and body condition. A body condition index of mass/standard length * 100 was calculated for admit and release to estimate overall body condition (Fadely, 1997).

2.2. Blood analysis

Blood samples (2–4 mL) were collected approximately every 2 weeks depending on clinical requirements. Samples were collected before the first feed of the day after an overnight fast. Animals were manually restrained and blood was collected via the extradural intravertebral vein (Bossart et al., 2001) into serum separator tubes (SST). Blood in SST was allowed to clot for 15 min then centrifuged at 3000×g for 10 min. Sera were collected, frozen, and shipped on dry ice to the University of North Florida and maintained at −80 °C until hormone analyses were completed. Due to differences in date of blood samples between animals, samples were grouped into two week periods based on the number of days since admittance: week 0 (<7 days since admit), week 2 (7–20 days), week 4 (21–34 days), week 6 (35–48 days), week 8 (49–63 days).

Growth hormone and IGF-I were quantified in the serum via heterologous radioimmunoassay (RIA) developed and validated for use with serum of multiple pinniped species by Richmond and Zinn (2009). Validations performed for NES pooled serum showed percentage recovery of growth hormone at 99.1 ± 8.0%. NES pooled serum averaged 11.0 ± 0.2 ng/ml GH and a dilution linearity with R^2 = 0.94. GH assays had an intra- and inter-assay coefficient of variation (CV) of 4.2 and 14.3 respectively. Insulin-like growth factor-I concentrations in pooled serum averaged 44.6 ± 1.7 ng/ml with a dilution linearity of R^2 = 0.94, an intra-assay CV of 6.6 and an inter-assay CV of 15.8. IGF-I assays had a detection limit of 20 ng/ml. Samples with concentrations below detection limits were included for statistical analysis at 10 ng/ml.