

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

Julie P. Richmond^{a,*}, Tenaya Norris^b, Steven A. Zinn^a

^aDepartment of Animal Science, University of Connecticut, 3636 Horsebarn Road Ext., Unit 4040, Storrs, CT 06269, USA

^bThe Marine Mammal Center, Marin Headlands, 2000 Bunker Road, Fort Cronkhite, Sausalito, CA 94965, USA

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 somatotrophic 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 somatotrophic 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 somatotrophic axis to varied levels of intake. These data suggest that multiple components of the somatotrophic 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.

© 2009 Elsevier Inc. All rights reserved.

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 somatotrophic 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 somatotrophic 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 somatotrophic 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 somatotrophic 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 somatotrophic 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 somatotrophic 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

* Corresponding author. Fax: +1 860 486 4375.

E-mail address: JULIE.RICHMOND@UCONN.EDU (J.P. Richmond).

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 somatotrophic 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 somatotrophic 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 3000g 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 ¹²⁵I-labeled IGF-I (GE Healthcare Bio-Sciences Corporation, Piscataway, NJ). After incubation, membranes were washed to remove unbound ¹²⁵I-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 mm² 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 IGFBP-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 $P \leq 0.05$ and trends if $0.10 > P > 0.05$. A stepwise linear regression analysis was conducted to assess the relationship between

hormone variables and body mass. Variables were retained in the model if $\alpha \leq 0.15$.

3. Results

Pups arrived at the rehabilitation facility thin and had been fasting for one to several days (nutritional nadir). During rehabilitation feed intake generally increased ($P < 0.01$; Fig. 1a). However, caloric intake declined slightly as animals were weaned from formula to fish. Intake was greater in 2005 compared with 2006 ($P < 0.01$; Fig. 2b). Pups gained body mass throughout rehabilitation ($P < 0.001$; Fig. 3a); however, 2005 pups gained more body weight at a faster rate compared with pups in 2006 ($P < 0.01$; Fig. 3b). Pups in 2005 gained $0.16 \pm 0.02 \text{ kg d}^{-1}$ while 2006 pups gained $0.10 \pm 0.02 \text{ kg d}^{-1}$ representing a 2% and 1% gain of body mass per day, respectively ($P = 0.03$; Table 1). Standard length increased an average of 10 cm over the 8 weeks of rehabilitation ($P < 0.01$; $71.8 \pm 1.4 \text{ cm}$ to $81.6 \pm 1.9 \text{ cm}$; week 1 and 9, respectively; Fig. 3a). There was no difference in standard length between years ($P = 0.13$) and no significant interaction between year and week of rehabilitation ($P = 0.57$). In 2006, axillary girth increased ($P < 0.001$; Fig. 3a) and condition index improved with rehabilitation ($P = 0.05$; Fig. 3b). The condition index increased from 71.5 ± 1.6 to 81.4 ± 3.4 between week 1 and 11 ($P = 0.01$; Fig. 3b).

When pups arrived at the facility males and females had similar body mass ($P = 0.76$; $8.1 \pm 0.5 \text{ kg}$); however, by week 7 males were

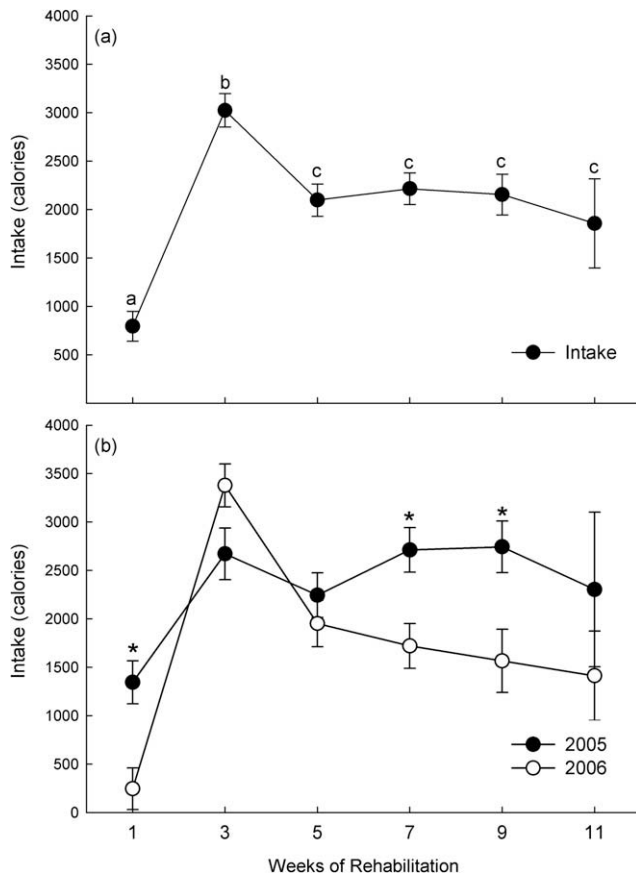


Fig. 1. Mean caloric intake of 28 harbor seal pups (19 female, 9 male) throughout rehabilitation. Most pups were weaned from formula to fish around week 4 of rehabilitation. Graph (a) illustrates the intake for all pups with in the study, and graph (b) separates intake by year of the study. Intake was greater in 2005 compared with 2006 ($P < 0.01$). Values shown are mean \pm SE. Unique letters identify statistical difference observed by week of rehabilitation, and (*) identify significant annual difference within week.

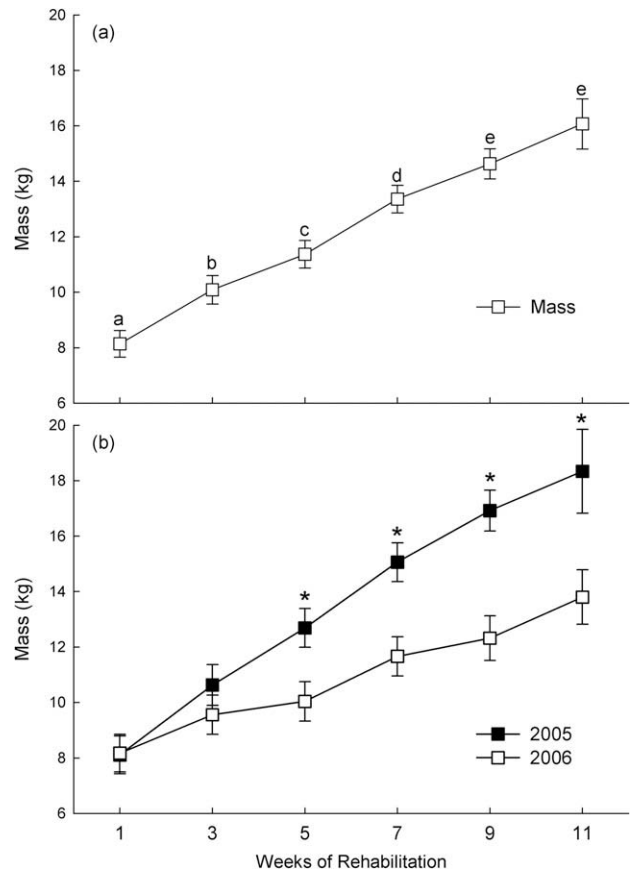


Fig. 2. Body mass of 28 harbor seal pups (19 female, 9 male) throughout rehabilitation and re-alimentation. Average mass per week of rehabilitation of all pups is illustrated in graph (a), and separated by year in graph (b). Pups gained body mass at a faster rate in 2005 compared with 2006 ($P < 0.01$). Values shown are mean \pm SE. Unique letters identify statistical difference observed by week of rehabilitation, and (*) identify significant annual difference within week.

larger than females ($P = 0.01$; 15.2 ± 0.9 vs. $12.5 \pm 0.6 \text{ kg}$, respectively; Table 1). Males grew at a faster rate ($P = 0.04$) compared with females (0.16 ± 0.02 vs. $0.10 \pm 0.02 \text{ kg d}^{-1}$, respectively; Table 1). There was a trend for male pups to consume more than females ($P = 0.07$; 1.3 ± 0.8 vs. $1.1 \pm 0.5 \text{ kg d}^{-1}$, respectively; Table 1).

Concentrations of GH (Fig. 4a) were greatest at nutritional nadir ($8.0 \pm 0.52 \text{ ng ml}^{-1}$, week 1) and then decreased with re-alimentation ($2.2 \pm 0.77 \text{ ng ml}^{-1}$, week 9; $P < 0.01$). Throughout rehabilitation, concentrations of GH were greater in 2006 compared with 2005 ($P = 0.01$; Fig. 4b). In contrast to the pattern observed in GH concentrations, IGF-I concentrations were reduced when pups arrived at rehabilitation facilities and increased with re-alimentation ($46 \pm 11.0 \text{ ng ml}^{-1}$, week 1; $151 \pm 13.7 \text{ ng ml}^{-1}$, week 9; $P < 0.001$; Fig. 4c). The slight decline in IGF-I observed at week 11 was not significant ($P = 0.13$) and was probably due to the release of larger pups before week 11. Parallel with feed intake and change in body mass, the 2006 IGF-I concentrations were less than concentrations observed in 2005 ($P = 0.05$; Fig. 4d). Concentrations of IGFBP-3 increased ($P < 0.01$; Fig. 4e) while IGFBP-2 remained consistent ($P = 0.58$; Fig. 4g) throughout rehabilitation. In 2005, IGFBP-3 increased at week 3 and then remained consistent (Fig. 4f). In contrast, 2006 concentrations of IGFBP-3 increased at week 3, but then declined back to concentrations similar to week 0 and remained less than 2005 concentrations throughout the remainder of rehabilitation ($P = 0.06$; Fig. 4f). Concentrations of IGFBP-2 in 2006 were consistent throughout rehabilitation while 2005 IGFBP-2 declined ($P < 0.001$; Fig. 4h). Overall concentrations of

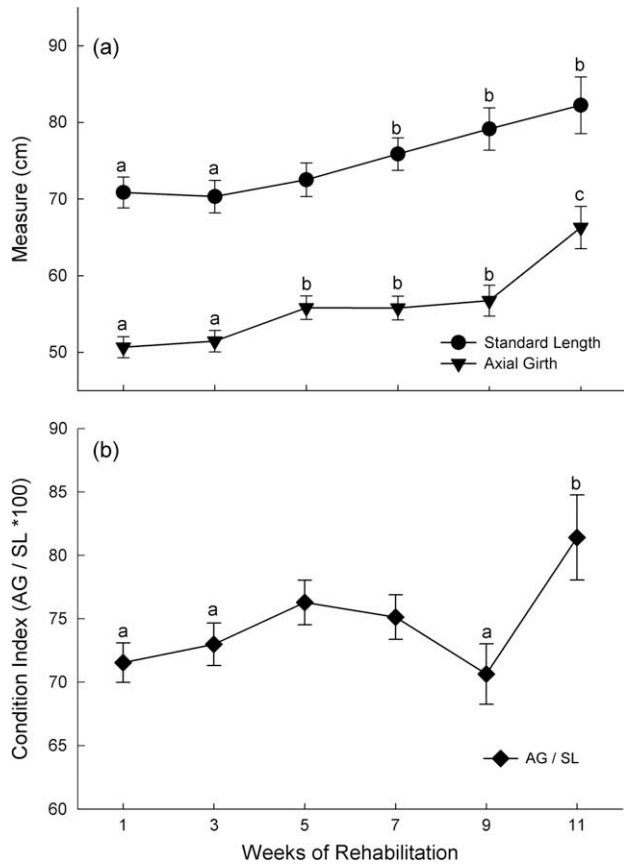


Fig. 3. Standard length (SL), axillary girth (AG), and condition index of 14 harbor seal pups (8 female, 6 male) throughout rehabilitation and re-alimentation in 2006. Values shown are mean ± SE. Unique letters within a specific measure identify statistical difference observed by week of rehabilitation.

IGFBP-2 were greater in 2005 compared with 2006 ($P < 0.001$). Female pups had greater overall concentrations of IGFBP-2 ($P = 0.01$; 85.2 ± 8.1 AU) compared with males (40.5 ± 13.4 AU). All hormones measured explained 70% of the variability in body mass as assessed by stepwise linear regression ($\text{mass} = 10.06 + 0.025 * \text{IGF-I} + 0.011 * \text{IGFBP-3} - 0.33 * \text{GH} - 0.012 * \text{IGFBP-2}$). Concentrations of IGF-I ($R^2 = 0.56$) and IGFBP-3 ($R^2 = 0.02$) were positively correlated with body mass, while GH ($R^2 = 0.10$) and IGFBP-2 ($R^2 = 0.02$) were negatively correlated with body mass.

Table 1

Gender and year differences in mean intake, morphometrics, and hormone concentrations of rehabilitated harbor seal pups.

	Gender		Year	
	Males	Females	2005	2006
Intake (kg d^{-1}) [*]	1.3 ± 0.8 ^x	1.1 ± 0.5 ^y	1.4 ± 0.8 ^a	1.0 ± 0.8 ^b
Calories (cal d^{-1})	2542 ± 196 ^x	2102 ± 131 ^y	2610 ± 194 ^a	2034 ± 139 ^b
Mass (kg) [†]	13.6 ± 0.8 ^x	11.8 ± 0.5 ^y	14.2 ± 0.7 ^a	11.1 ± 0.6 ^b
Growth rate (kg d^{-1})	0.16 ± 0.02 ^a	0.10 ± 0.02 ^b	0.16 ± 0.02 ^a	0.10 ± 0.02 ^b
Standard length (cm)	74.7 ± 2.2	75.2 ± 1.6	79.3 ± 2.7 ^a	75.2 ± 1.7
Axillary girth (cm)	57.7 ± 1.5	54.0 ± 1.3	ND	55.8 ± 1.0
Condition index	75.2 ± 1.3	74.2 ± 1.2	ND	74.7 ± 0.9
GH (ng ml^{-1})	3.4 ± 0.7	3.8 ± 0.5	2.5 ± 0.6 ^a	4.7 ± 0.6 ^b
IGF-I (ng ml^{-1})	101.3 ± 15.3	103.8 ± 10.6	128.7 ± 12.9 ^a	77.1 ± 12.1 ^b
IGFBP-3 (AU)	124.2 ± 11.3	101.4 ± 16.8	137.0 ± 13.7 ^a	98.1 ± 14.1 ^b
IGFBP-2 (AU)	40.5 ± 13.4 ^a	85.2 ± 8.1 ^b	95.9 ± 11.3 ^a	29.9 ± 10.0 ^b

Hormone concentrations measured include growth hormone (GH), insulin-like growth factor (IGF-I), and insulin-like growth factor binding proteins (IGFBP). Concentrations of IGFBP are shown in arbitrary units (AU). Condition index was calculated as axillary girth/standard length × 100 (Fadely, 1997). Values shown are mean ± SE. Different letters within gender or year indicate significant difference at $P \leq 0.05$ (a, b) or trend if $0.05 < P < 0.01$ (x, y). ND indicates no data collected.

^{*} A trend was observed for the interaction of gender and year for intake ($P = 0.073$).

[†] There was a significant interaction between gender and year for mass ($P = 0.013$).

4. Discussion

In this study, the influence of nutrition (reduced nutrient intake and re-alimentation), on the response of the somatotrophic axis and growth rate was examined in harbor seal pups. Harbor seals are a species commonly rehabilitated across North America and successfully released back into the wild (NMFS National Stranding Database). Harbor seal pups may strand for many reasons, including premature separation of mother-pup pairs, maternal abandonment, sickness, injuries and human interference (Gage, 2002). Proper body weight gain during the rehabilitation process is an important factor for post-release survival (Lander et al., 2002). Stranded harbor seal pups provide a unique model to study the response of the somatotrophic axis to changes in nutritional status because they arrive in rehabilitation facilities at a low plane of nutrition.

Since pups had been fasting for several days, our initial sample represents a nutritional nadir, and likely illustrates an extremely low plane of nutrition relative to healthy free-ranging animals of a similar age (Richmond et al., 2008). Intake was initially low and gradually increased. Intake plateaued after 5 weeks of rehabilitation when all pups were weaned to fish. After 5 weeks, intake remained consistent at around 2000 cal d^{-1} .

In the wild, pups average 8–12 kg at birth and typically nurse for 4–6 weeks on high fat milk (50% lipid), and gain an average of $0.6\text{--}0.7 \text{ kg d}^{-1}$ throughout nursing, which corresponds to 5–9% daily increases in body mass (Bowen et al., 2001b; Schulz and Bowen, 2004; Lang et al., 2005). Here pups were fed formula consisting of approximately 36% fat and weaned around 5 weeks of age, after 4 weeks of rehabilitation. At the start of rehabilitation all pups were of similar body mass ($8.1 \pm 0.5 \text{ kg}$), regardless of year or gender. All pups gained mass through out rehabilitation, but at a much slower rate (<2% per day) than animals in the wild.

Consistent with patterns observed in domestic and wild carnivores, GH was greatest when pups were at nutritional nadir (Eigenmann et al., 1985; Nieminen et al., 2004). In domestic species, elevated GH spares body protein and facilitates the use of adipose stores for maintenance energy needs (Breier, 1999). As intake increased, GH declined and plateaued, attaining values similar to free-ranging harbor seal pups of comparable age (Richmond et al., 2008).

In contrast, IGF-I concentrations were decreased at the onset of rehabilitation and gradually increased as re-alimentation occurred. Similar patterns in IGF-I concentration have been observed in dogs, cats, and pigs with reduced concentrations during prolonged food

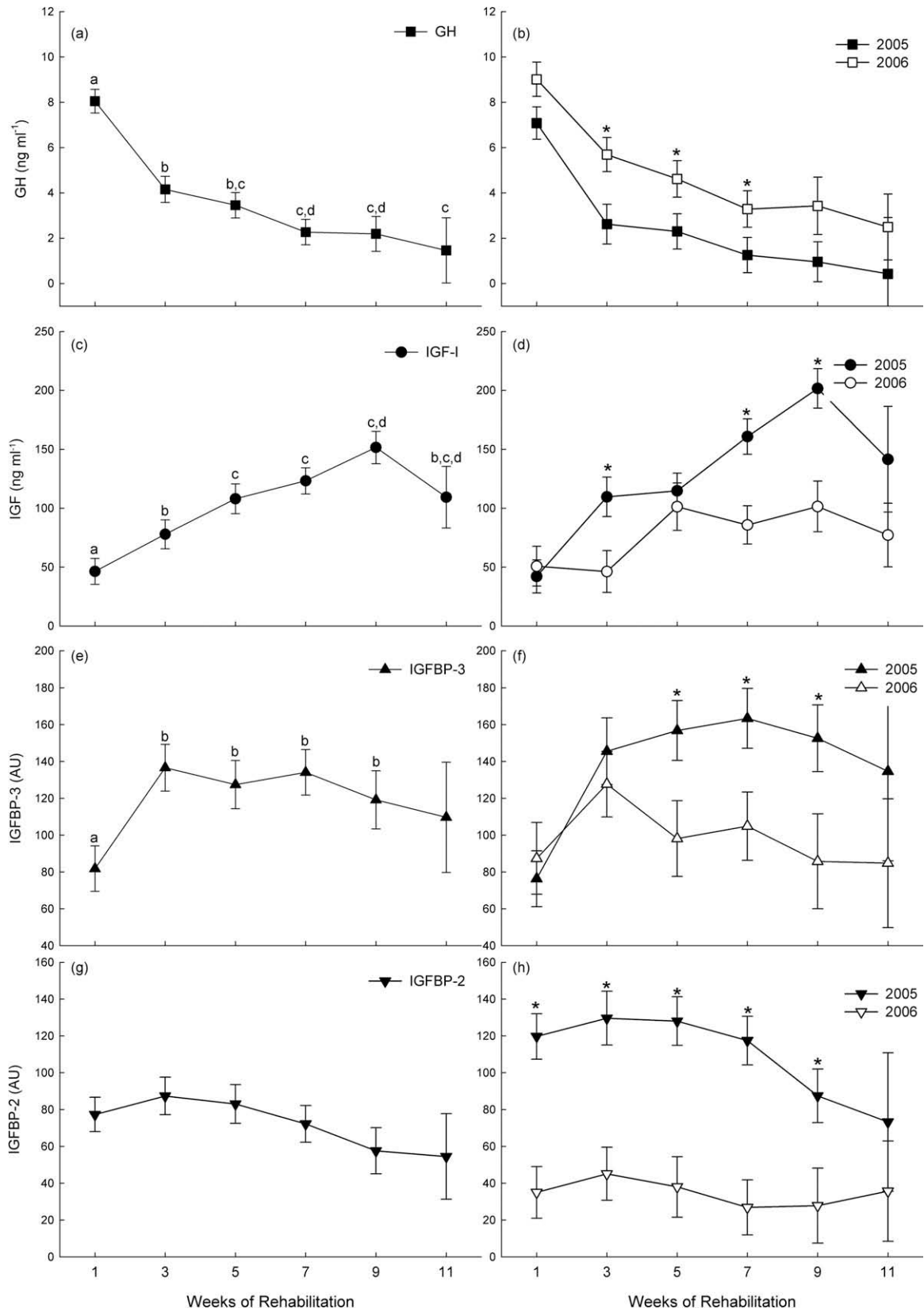


Fig. 4. Changes in growth hormone (GH), insulin-like growth factor (IGF-I), and insulin-like growth factor binding proteins (IGFBP) of 28 harbor seal pups (19 female, 9 male) throughout rehabilitation and re-alimentation. Graphs (a, c, e, and g) illustrate average concentrations for all pups, and graphs (b, d, f, and h) separate concentrations by year of the study. Pups had greater IGF-I, IGFBP-2 and IGFBP-3 concentrations, and reduced GH concentrations in 2005 compared with 2006 ($P < 0.05$). Values shown are mean \pm SE. Unique letters within a measure identify statistical difference observed by week of rehabilitation, and (*) identify significant annual difference within week.

restriction and recovery of IGF-I concentrations following two or more weeks of re-feeding (Maxwell et al., 1998, 1999; Sohlström et al., 1998). Concentrations of IGF-I are often low in nutritional deficit to spare nutrients from lean tissue growth so that energy can be allocated for maintenance energy needs (Gautsch et al., 1998). The changes in IGF-I with changes in nutrient intake and associated changes in growth rate (i.e., increased nutrient intake, increased IGF-I, and increased growth rate, or reduced nutrient intake, decreased IGF-I and decreased growth rate) illustrates the role IGF-I plays in modulating the response of the somatotrophic axis to nutrient intake resulting in changes in growth rate.

Concentration of IGFBP also responded dramatically to increased nutrient intake. There was almost a 2-fold increase in IGFBP-3 concentration between week 1 and 3 of rehabilitation. After which IGFBP-3 concentration remained relatively constant. In most mammals, IGFBP-3 is positively associated with intake and growth rate, and typically mirrors the pattern of IGF-I expression (Jones and Clemmons, 1995; Maxwell et al., 1998). The greatest increase in harbor seal IGFBP-3 was observed during the initial re-feeding when caloric intake increased 5-fold. As rehabilitation progressed and intake modulated so did IGFBP-3 concentrations. In harbor seals, IGFBP-3 appears to be closely associated with energy intake, while IGF-I concentrations reflected changes in mass.

In contrast to the pattern observed in IGFBP-3 with re-alimentation, IGFBP-2 concentrations gradually declined with re-alimentation (observed in 2005, but not 2006). Increased IGFBP-2 is observed in feed restricted canines (Maxwell et al., 1998) and is negatively associated with growth rate in most species, although response can vary by species (Nap et al., 1993; Govoni et al., 2003). In most mammals, IGFBP-2 is thought to have an inhibitory effect on IGF-I interaction with target tissues (Jones and Clemmons 1995). This pattern of change (i.e., increased GH and IGFBP-2 and decreased IGF-I and IGFBP-3 in low nutritional state; compared with decreased concentrations of GH and IGFBP-2 and increased IGF-I and IGFBP-3 when individuals were at a higher plane of nutrition) are characteristic of the typical mammalian response of the somatotrophic axis to changes in nutrient intake (Gautsch et al., 1998; Maxwell et al., 1998; Rausch et al., 2002; Richmond et al., 2008).

Whereas a direct measure of post-release survival was not conducted in this study, other research has shown that a greater mass at weaning results in increased first year survival of harbor seal pups (Muelbert et al., 2003; Harding et al., 2005). Although, rehabilitated pups were still smaller than comparable free-ranging pups of similar age, the increased mass at release may confer an advantage for the larger rehabilitated pups. Increased size may provide larger pups with greater reserves for maintenance energy until foraging skills can improve to provide sufficient intake for maintenance and growth (Noren et al., 2008). Evaluation of the somatotrophic axis in rehabilitated seals may provide an assessment tool to evaluate rehabilitation success. Further, the evaluation of the somatotrophic axis in free-ranging pups may provide a physiologic measure that can assess the effect of changes in nutritional status of an individual that influences growth rate and hence survival.

Although all pups initiated rehabilitation at a similar mass, males had a great growth rate leading to greater overall body mass compared with female pups. In the wild, male pups are larger at birth and weaning compared with females, but growth rates are similar (Bowen et al., 2001a). The differential growth rate observed in this study may suggest that male pups have a greater capacity for compensatory growth than females after food restriction (Hornick et al., 2000). Similar to domestic species, female harbor seal pups had greater concentrations of IGFBP-2 compared with males. Since IGFBP-2 is negatively associated with growth rate (Govoni et al., 2003), the increased concentration of IGFBP-2 in females

may have contributed to their reduced growth rate, relative to males, especially given that all other hormone concentrations measured were similar between males and females.

The inter-annual differences observed were unexpected, but clearly illustrate the response of the somatotrophic axis to varying levels of nutrient intake and resulting differences in growth rate. In 2005, pups were fed more (kg of fish) and had a greater overall caloric intake. The caloric intake for pups was estimated assuming batches of fish were of similar caloric content. The energy content of herring can vary dramatically depending on age, season, and size (Payne et al., 1999). It is not unusual to see batches of herring vary by 1000 cal kg⁻¹ or more. While pups in this study were all fed herring at a rate of approximately 10% body mass, overall caloric intake, using estimates of caloric value for herring, was greater in 2005 compared with 2006. Therefore, differences in energy content of the batches of fish fed in the two years may have further increased the actual deviation in caloric intake between years. That is, actual energy intake in 2006 may have been even less than what was estimated. Regardless of the magnitude of the differences in intake, the differential caloric intake between 2005 and 2006 resulted in differential growth rates. As a result, there was an unexpected treatment manipulation that provided an opportunity to examine the response of the somatotrophic axis to re-alimentation as originally designed, and also examine the differential response in growth rate and metabolic hormones to differential rates of feed intake. Although intake rates and year of rehabilitation are confounded, these data provide important information on growth rate and the endocrine response to the level of feed intake.

In 2006, pups with less caloric intake gained mass at a slower rate compared with animals in 2005. Because axillary girth was only measured in 2006, an assessment of condition index between years was not possible. However, animals in 2005 had a greater mass, but similar standard length to pups in 2006, suggesting that the increase in mass was not due to linear growth, but more likely due to increased body condition (Fadely, 1997). This differential intake and growth rate was also reflected in the response of the somatotrophic axis. Concentrations of GH were less and IGF-I, IGFBP-3, and IGFBP-2 were all greater in 2005 compared with 2006. These differences in hormone concentration resulted in a greater growth rate in 2005 and greater mass at release. As in domestic species, the magnitude of the response of the somatotrophic axis was related to the magnitude of the growth response in harbor seals (Govoni et al., 2003). Since both caloric intake and growth rate are greater in free-ranging pups compared with rehabilitated pups, it may be expected that the magnitude of the response of the somatotrophic axis to nutrient intake in free-ranging pups may be even greater than in the current study. Further, the somatotrophic axis may be used to predict growth response of individuals in the wild.

5. Conclusion

In support of our hypothesis, the somatotrophic axis in harbor seals responded to nutrient deficit and re-alimentation as predicted based on the domestic animal model. When animals arrived at TMMC they were thin and had likely been fasting for one to several days. At this nutritional nadir, IGF-I and IGFBP-3 were decreased, while GH and IGFBP-2 were elevated. Throughout the re-alimentation process pups moved into a positive energy balance. As nutrient intake increased, GH and IGFBP-2 declined while IGF-I and IGFBP-3 increased, which was associated with gain in total body mass and increased body condition. These trends were similar to our predictions based on the domestic animal model. Further, the magnitude of the growth response was related to the magnitude in response of the components of the somatotrophic axis to varied levels of intake. These data suggest that the hormone

profile of the somatotrophic 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. By investigating physiological factors that link nutrition and growth, we can assess the impact of decreased nutrient intake on growth and body composition that may have implications for survival.

Acknowledgments

The authors thank the rehabilitation staff and volunteers at The Marine Mammal Center, especially F. Gulland and D. Wickham for their support of this project and comments on earlier versions of the manuscript. We also thank L. Mazzaro from the Mystic Aquarium & Institute for Exploration for facilitating the project and G. Comeau from the University of Connecticut for laboratory support. Funding for this research was provided by the University of Connecticut Research Foundation and USDA-Hatch. Samples were collected as a part of routine veterinary care and were authorized under a Letter of Authorization from National Marine Fisheries Service for The Marine Mammal Center (EAJ 1514-10). Protocols were reviewed and approved by IACUC at the University of Connecticut (E05-115).

References

- Bossart, G.D., Reidarson, T.H., Dierauf, L.A., Duffield, D.A., 2001. Clinical pathology. In: Dierauf, L.A., Gulland, F.M. (Eds.), CRC Handbook of Marine Mammal Medicine. CRC Press, Boca Raton, FL, pp. 383–390.
- Boulva, J., 1975. Temporal variations in birth periods and characteristics of newborn harbour seals. Rapp. Proc. Verba. Reun. 169, 405–408.
- Bowen, W.D., Ellis, S.L., Iverson, S.J., Boness, D.J., 2001a. Maternal effects on offspring growth rate and weaning mass in harbour seals. Can. J. Zool. 79 (6), 1088–1101.
- Bowen, W.D., Iverson, S.J., Boness, D.J., Oftedal, O.T., 2001b. Foraging effort, food intake and lactation performance depend on maternal mass in a small phocid seal. Funct. Ecol. 15 (3), 325–334.
- Bowen, W.D., Oftedal, O.T., Boness, D.J., Iverson, S.J., 1994. The effect of maternal age and other factors on birth mass in the harbour seal. Can. J. Zool. 72 (1), 8–14.
- Breier, B.H., 1999. Regulation of protein and energy metabolism by the somatotrophic axis. Domest. Anim. Endocrinol. 17 (2–3), 209–218.
- Burns, J.J., 2002. Harbor seal and spotted seal (*Phoca vitulina* and *P. largha*). In: Perrin, W.F., Würsig, B., Thewissen, J.G.M. (Eds.), Encyclopedia of Marine Mammals. Academic Press, San Diego, California, pp. 552–560.
- Burns, J.M., Clark, C.A., Richmond, J.P., 2005. Is hematological development in juvenile pinnipeds limited by iron intake? FASEB J. 19 (4), A672.
- Connor, E.E., Barao, S.M., Kimrey, A.S., Parlier, A.B., Douglass, L.W., Dahl, G.E., 2000. Predicting growth in angus bulls: the use of GHRH challenge, insulin-like growth factor-I, and insulin-like growth factor binding proteins. J. Anim. Sci. 78 (11), 2913–2918.
- Eigenmann, J.E., de Bruijne, J.J., Froesch, E.R., 1985. Insulin-like growth factor I and growth hormone in canine starvation. Acta. Endocrinol. Copenhagen. 108 (2), 161–166.
- Eisert, R., Oftedal, O.T., Lever, M., Ramdohr, S., Breier, B.H., Barrell, G.K., 2005. Detection of food intake in a marine mammal using marine osmolytes and their analogues as dietary biomarkers. Mar. Ecol. Prog. Ser. 300, 213–228.
- Fadely, B.S., 1997. Investigations of health status and body condition of harbor seals (*Phoca vitulina*) in the Gulf of Alaska. Ph.D. Dissertation. University of Alaska Fairbanks, Fairbanks, Alaska, pp. 183.
- Freake, H.C., Govoni, K.E., Guda, K., Huang, C., Zinn, S.A., 2001. Actions and interactions of thyroid hormone and zinc status in growing rats. J. Nutr. 131 (4), 1135–1141.
- Gage, L.J., 2002. Hand-rearing Wild and Domestic Mammals. Iowa State Press, Ames, Iowa.
- Gautsch, T.A., Kandl, S.M., Donovan, S.M., Layman, D.K., 1998. Response of the IGF-I system to prolonged undernutrition and its involvement in somatic and skeletal muscle growth retardation in rats. Growth Dev. Aging 62 (1–2), 13–25.
- Gill, J.L., Hafs, H.D., 1971. Analysis of repeated measurements of animals. J. Anim. Sci. 33 (2), 331–336.
- Govoni, K.E., Hoagland, T.A., Zinn, S.A., 2003. The ontogeny of the somatotrophic axis in male and female Hereford calves from birth to one year of age. J. Anim. Sci. 81 (11), 2811–2817.
- Gulland, F.M.D., Lowenstine, L.J., Lapointe, J.M., Spraker, T.R., King, D., 1997. Herpesvirus infection in Pacific harbour seals (*Phoca vitulina richardsii*) stranded along the central California coast. J. Wildl. Dis. 33, 450–458.
- Harding, K.C., Fujiwara, M., Axberg, Y., Härkönen, T., 2005. Mass-dependent energetics and survival in harbour seal pups. Funct. Ecol. 19 (1), 129–135.
- Harrell, R.J., Thomas, M.J., Boyd, R.D., Czerwinski, S.M., Steele, N.C., Bauman, D.E., 1999. Ontogenic maturation of the somatotropin/insulin-like growth factor axis. J. Anim. Sci. 77 (11), 2934–2941.
- Hornick, J.L., Van Eenaeme, C., Gérard, O., Dufresne, I., Istasse, L., 2000. Mechanisms of reduced and compensatory growth. Dom. Anim. Endo. 19 (2), 121–132.
- Jones, J.L., Clemmons, D.R., 1995. Insulin-like growth factors and their binding proteins: biological actions. Endocr. Rev. 16 (1), 3–34.
- Lander, M.E., Harvey, J.T., Gulland, F.M.D., 2003. Hematology and serum chemistry comparisons between free-ranging and rehabilitated harbor seal (*Phoca vitulina richardsii*) pups. J. Wildl. Dis. 39 (3), 600–609.
- Lander, M.E., Harvey, J.T., Hanni, K.D., Morgan, L.E., 2002. Behavior, movements, and apparent survival of rehabilitated and free-ranging harbor seal pups. J. Wildl. Manag. 66 (1), 19–28.
- Lang, S.L.C., Iverson, S.J., Bowen, W.D., 2005. Individual variation in milk composition over lactation in harbour seals (*Phoca vitulina*) and the potential consequences of intermittent attendance. Can. J. Zool. 83 (12), 1525–1531.
- Lawrence, T.L.J., Fowler, V.R., 1997. Hormonal, genetic and immunological influences on growth. In: Growth of Farm Animals. Anonymous CAB International, London, pp. 102–149.
- Maxwell, A., Butterwick, R., Yateman, M., Batt, R., Cotterill, A., Camacho-Hubner, C., 1998. Nutritional modulation of canine insulin-like growth factors and their binding proteins. J. Endocrinol. 158 (1), 77–85.
- Maxwell, A., Butterwick, R., Batt, R.M., Camacho-Hubner, C., 1999. Serum insulin-like growth factor (IGF)-I concentrations are reduced by short-term dietary restriction and restored by refeeding in domestic cats (*Felis catus*). J. Nutr. 129 (10), 1879–1884.
- Muelbert, M.M., Bowen, W.D., Iverson, S.J., 2003. Weaning mass affects changes in body composition and food intake in harbour seal pups during the first month of independence. Physiol. Biochem. Zool. 76 (3), 418–427.
- Nap, R.C., Mol, J.A., Hazewinkel, H.A.W., 1993. Age-related plasma concentrations of growth hormone (GH) and insulin-like growth factor I (IGF-I) in great dane pups fed different dietary levels of protein. Dom. Anim. Endo. 10 (3), 237–247.
- Nieminen, P., Saarela, S., Pykönen, T., Asikainen, J., Mononen, J., Mustonen, A., 2004. Endocrine response to fasting in the overwintering captive raccoon dog (*Nyctereutes procyonoides*). J. Exp. Zool. 301A (12), 919–929.
- Noren, S.R., Boness, D.J., Iverson, S.J., McMillan, J., Bowen, W.D., 2008. Body condition at weaning affects the duration of the postweaning fast in gray seal pups (*Halichoerus grypus*). Physiol. Biochem. Zool. 81 (3), 269–277.
- Ortiz, R.M., Noren, D.P., Ortiz, C.L., Talamantes, F., 2003. GH and ghrelin increase with fasting in a naturally adapted species, the northern elephant seal (*Mirounga angustirostris*). J. Endocrinol. 178 (3), 533–539.
- Payne, S.A., Johnson, B.A., Otto, R.S., 1999. Proximate composition of some north-eastern Pacific forage fish species. Fish. Oceanogr. 8, 159–177.
- Rausch, M.I., Tripp, M.W., Govoni, K.E., Zang, W., Weibert, W.J., Crooker, B.A., Hoagland, T.A., Zinn, S.A., 2002. The influence of level of feeding on growth and serum insulin-like growth factor I and insulin-like growth factor-binding proteins in growing beef cattle supplemented with somatotropin. J. Anim. Sci. 80 (1), 94–100.
- Renaville, R., Van Eenaeme, C., Breier, B.H., Vleurick, L., Bertozzi, C., Gengler, N., Hornick, J.L., Parmentier, I., Istasse, L., Haezebroeck, V., Massart, S., Portetelle, D., 2000. Feed restriction in young bulls alters the onset of puberty in relationship with plasma insulin-like growth factor-I (IGF-I) and IGF-binding proteins. Domest. Anim. Endocrinol. 18 (2), 165–176.
- Richmond, J.P., Zinn, S.A., 2009. Validation of radioimmunoassays (RIA) for growth hormone (GH) and insulin-like growth factor (IGF)-I in phocid, otariid, and cetacean species. Aquat. Mamm. 35 (1), 19–31.
- Richmond, J.P., Skinner, J., Gilbert, J., Mazzaro, L.M., Zinn, S.A., 2008. Comparison of the somatotrophic axis in free-ranging and rehabilitated harbor seal pups (*Phoca vitulina*). J. Zoo Wildl. Med. 39 (3), 342–348.
- Sohlström, A., Katsman, A., Kind, K.L., Grant, P.A., Owens, P.C., Robinson, J.S., Owens, J.A., 1998. Effects of acute and chronic food restriction on the insulin-like growth factor axis in the guinea pig. J. Endocrinol. 157 (1), 107–114.
- Schulz, T.M., Bowen, W.D., 2004. Pinniped lactation strategies: evaluation of data on maternal and offspring life history traits. Mar. Mamm. Sci. 20 (1), 86–114.
- Smith, W.J., Underwood, L.E., Clemmons, D.R., 1995. Effects of caloric or protein restriction on insulin-like growth factor-I (IGF-I) and IGF-binding proteins in children and adults. J. Clin. Endocrinol. Metab. 80 (2), 443–449.
- Straus, D.S., 1994. Nutritional regulation of hormones and growth factors that control mammalian growth. FASEB J. 8 (1), 6–12.
- Straus, D.S., Takemoto, C.D., 1990. Effect of dietary protein deprivation on insulin-like growth factor (IGF)-I and -II, IGF binding protein-2, and serum albumin gene expression in rat. Endocrinology 127 (4), 1849–1860.