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Seasonal Influence on the Response of the Somatotropic Axis to Nutrient Restriction and Re-alimentation in Captive Steller Sea Lions (*Eumetopias jubatus*)

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ABSTRACT Fluctuations in availability of prey resources can impede acquisition of sufficient energy for maintenance and growth. By investigating the hormonal mechanisms of the somatotropic axis that link nutrition, fat metabolism, and lean tissue accretion, we can assess the physiological impact of decreased nutrient intake on growth. Further, species that undergo seasonal periods of reduced intake as a part of their normal life history may have a differential seasonal response to nutrient restriction. This experiment evaluated the influence of season and age on the response of the somatotropic axis, including growth hormone (GH), insulin-like growth factor (IGF)-I, and IGF-binding proteins (BP), to reduced nutrient intake and re-alimentation in Steller sea lions. Eight captive females (five juveniles, three sub-adults) were subject to 28-day periods of food restriction, controlled re-feeding, and ad libitum recovery in summer (long-day photoperiod) and winter (short-day photoperiod). Hormone concentrations were insensitive to type of fish fed (low fat pollock vs. high fat herring), but sensitive to energy intake. Body mass, fat, and IGF-I declined, whereas GH and IGFBP-2 increased during feed restriction. Reduced IGF-I and IGFBP with increased GH during controlled re-feeding suggest that animals did not reach positive energy balance until fed ad libitum. Increased IGF-I, IGFBP-2, IGFBP-3, and reduced GH observed in summer reflected seasonal differences in energy partitioning. There was a strong season and age effect in the response to restriction and re-alimentation, indicating that older, larger animals are better able to cope with stress associated with energy deficit, regardless of season. *J. Exp. Zool. 313A:144–156, 2010.* © 2010 Wiley-Liss, Inc.

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The 80% decline in Steller sea lion (*Eumetopias jubatus*) populations between 1970 and 2000 has stimulated significant research to determine the cause of the decline (Trites and Larkin, '96; Atkinson et al., 2008b). Although many plausible explanations have been put forth, nutritional stress remains at the forefront of hypotheses under investigation for both the original cause of the decline and current failure of populations to recover (Atkinson et al., 2008b). Decreased quantity or quality of prey resources, leading to nutritional stress, may be especially

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detrimental to juvenile survival (Trillmich and Ono, '91; Horning and Trillmich, '99; Österblom et al., 2008). Young pinnipeds, like most developing mammals, need to maintain an elevated growth rate to attain mature size, yet their lack of foraging experience (Rehberg and Burns, 2008) and immature physiological development (Richmond et al., 2005, 2006) may limit their ability to attain sufficient energy intake in suboptimal conditions, which could reduce overall growth (Horning and Trillmich, '99; Winship et al., 2001; Pitcher et al., 2005). Decreased growth rate resulting in a smaller size may ultimately lead to decreased survival (Muelbert et al., 2003). Further exacerbating the challenges of acquiring sufficient nutrients for growth in Steller sea lions are fluctuations in availability of prey resources (Sinclair and Zeppelin, 2002; Gende and Sigler, 2006; Womble and Sigler, 2006) and life history traits that include extended periods of fasting on shore during the summer breeding season (Gentry, '70; Higgins et al., '88; Rea et al., '98; Maniscalco et al., 2006). Although Steller sea lions are naturally subject to seasonal variation in prey availability and intake (Raum-Suryan et al., 2004; Gende and Sigler, 2006; Maniscalco et al., 2006), captive studies that have imposed nutrient restrictions outside the normal life history pattern of the species have demonstrated that season appears to have a substantial effect on the impact of episodes of nutritional stress on body condition and physiology (Rosen and Trites, 2005; Kumagai et al., 2006; Rosen and Kumagai, 2008). Recent research has also shown that juvenile Steller sea lions, unlike sub-adults, show no metabolic adaptations to fasting which could put them at greater risk during a period of nutrient restriction compared with older animals (Rea et al., 2007, 2009).

Although recent research has furthered our understanding of the gross metabolic adjustments and changes in body composition that occurs in sea lions during periods of fasting (Rosen and Trites, 2002; Rea et al., 2007), or when fed a low energy diet (Rosen and Trites, '99; Rosen and Trites, 2005; Kumagai et al., 2006), little is known about the underlying endocrine mechanism by which decreased nutrient intake influences growth physiology of Steller sea lions. The role of the somatotropic axis as a bridge between growth physiology, development, and nutritional status has been well documented in domestic animals. However, the function of the somatotropic axis during periods of normal growth, nutrient restriction, and re-alimentation in marine mammals has not been previously explored. 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 a key modulator of nutrient allocation for accretion of protein and adipose (Lawrence and Fowler, '97). There are six IGFBP which regulate the biological actions of IGF-I (Jones and Clemmons, '95), but IGFBP-2 and IGFBP-3 are sensitive to nutrient intake and most closely associated with growth rate (Oster et al., '95; Harrell et al., '99; Rausch et al., 2002; Govoni et al., 2003).

The role of the somatotropic axis in cell proliferation and growth and the response to changes in nutrient intake are well documented in domestic species. With adequate nutrient intake, increased GH stimulates production of IGF-I in liver and peripheral tissues; however, in nutrient restriction, the stimulatory effect of GH on hepatic IGF-I production is uncoupled. 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 (Oster et al., '95; Maxwell et al., '98; Rausch et al., 2002). Increased concentrations of GH are observed during energy deficit due to decreased metabolic clearance rate of GH (Breier, '99) to mobilize energy reserves from adipose tissue and preserve body protein (Johnsson and Hart, '86). In contrast, IGF-I and IGFBP-3 concentrations decrease with inadequate nutrition, due in part to decreased GH sensitivity in the liver, where the majority of circulating IGF-I is produced (Straus, '94; Smith et al., '95). During limited nutrient availability, IGFBP-2 is increased, reducing availability of IGF-I for peripheral tissues (Renaville et al., 2000). Investigations of the response of the somatotropic axis to changes in nutrient intake in marine mammals has been limited, but studies with phocid (true seal) species suggest that they respond in a similar manner as terrestrial mammals when intake is restricted or increased (Ortiz et al., 2003; Eisert et al., 2005; Richmond et al., 2008).

Although these limited data suggest pinnipeds exhibit a typical mammalian hormonal response to natural variations in nutrient intake, longitudinal experiments investigating the effects of experimental nutrient restriction and re-alimentation have not been performed. Further, the recent determination that juveniles are not as efficient at protein sparing compared with sub-adults during periods of fasting, and that this ability is seasonally dependent, suggests that the physiological mechanism responsible for energy partitioning during periods of reduced intake may also be age dependent and seasonally regulated (Rea et al., 2007, 2009).

Therefore, we sought to investigate the effects of season and age on the response of the somatotropic axis to reduced nutrient intake and re-alimentation in Steller sea lions. Specifically we examined the response of the somatotropic axis and its relationship to body condition in eight female Steller sea lions subject to 28-day periods of food restriction, controlled re-feeding and ad libitum recovery. To determine whether the response of the somatotropic axis was seasonally dependent the experimental design was repeated with the same individuals in summer (long-day photoperiod) and winter (short-day photoperiod). Finally, as previous research has shown that juvenile Steller sea lions do not possess the same metabolic adaptations for fasting as sub-adult sea lions, we examined the differential response of juvenile (3-year-old) and sub-adult (6-yr-old) sea lions. Examining the changes in key metabolic hormones that underlie gross morphological changes will allow us to accurately understand the physiological consequences of changes in prey intake on Steller sea lion growth and ultimately survival.

MATERIALS AND METHODS

All animal procedures were approved by the University of British Columbia Animal Care Committee (No. A04-0169) and the University of Connecticut, Institutional Animal Care Committee (E05-115). This study was conducted in conjunction with other studies evaluating the seasonal response of Steller sea lions to nutritional stress (Jeanniard du Dot et al., 2008, 2009).

Animals and Diets

Eight Steller sea lion females ($n = 5$, 3-year-old juveniles and $n = 3$, 6-year-old sub-adults) housed at Vancouver Aquarium (VA; Vancouver, BC, Canada) were used to examine changes in the somatotropic axis in response to reduced nutrient intake and re-alimentation. Experiments were conducted with the same individuals in the summer (June–October, long-day photoperiod) and again in the following winter (January–April, short-day photoperiod) to evaluate the effects of season. The experiments were divided into four phases and animals were randomly assigned to one of two diet treatment groups. The diet treatments were identical except for Phase II. In Phase I, animals were fed ad libitum Pacific herring (*Clupea pallasi*) for 4 weeks. Animals consumed approximately 4.0 to 5.5% of their body mass. In Phase II, the restriction phase, four animals (three juveniles and one sub-adult) continued to eat the high fat herring (11% fat w.w.) but at a reduced quantity, and four animals (two juveniles and two sub-adults) were fed an isocaloric low fat (7% fat) Walleye pollock (*Theragra chalcogramma*) diet for 4 weeks. This restriction diet contained approximately 75% of the energy content of the Phase I ad libitum diet. The diets were designed so that animals would not lose more than 15% of their original body mass during Phase II. In Phase III, the controlled re-feeding phase, animals were fed herring at an energy intake equal to the original Phase I diet for an additional 4 weeks. During the summer experiment juveniles did not re-gain body mass as had been anticipated during Phase III. Therefore, an additional sample was collected from juveniles after they had been fed ad libitum for 6 weeks. In the winter experiment an additional 2-week phase (IV) of ad libitum herring feeding was added.

Proximate composition was determined for a subset of the fish fed (Northwest Labs, Surry, BC, Canada), and used to calculate gross energy content (Schmidt-Nielsen and Duke, ’97). Food intake and body mass were recorded daily for each sea lion. Blood collection, standard length measurements, and body composition determined by deuterium dilution (Reilly et al., ’96; Bowen et al., ’98) were performed at the end of Phase I and repeated every 2 weeks until the end of the experiment (i.e., start, middle, and end of each phase). All blood collections were completed in the morning before the first feeding after an overnight fast (approximately 18 hr) to minimize the effect of intake and diel variation on hormone concentrations. Body composition including total body fat and lean body mass were derived from body water estimates with equations from Arnould et al. (’96).

Blood Handling and Hormone Quantification

Blood was collected from a caudal gluteal vein (Bossart et al., 2001) into serum separator tubes while animals were under isoflurane gas anesthesia (Heath et al., ’97). Blood was centrifuged (959g for 5 min at 4°C) and sera were collected, frozen, and maintained at −70°C until processing. Heterologous radioimmunoassays (RIA) were used to quantify GH and IGF-I concentrations with methods previously validated for Steller sea lion serum (Richmond and Zinn, 2009). All anti-sera and standards were purchased from A. F. Parlow, National Hormone & Peptide Program (Torrance, CA). Rabbit-anti-porcine GH antisera (AFP422801Rb) was used with porcine GH standard curve (AFP10864B) to quantify sea lion GH, and IGF-I was quantified using a rabbit-anti-human antibody (AFP4892898) and human IGF-I standard (Lot#01). Detailed methods and validations were published in Richmond and Zinn (2009). Concentrations of IGFBP–2 and IGFBP–3 were determined by Western ligand blot (Freake et al., 2001). Serum proteins were separated by polyacrylamide gel electrophoresis, transferred to a nitrocellulose membrane and incubated overnight with approximately 1.6 MBq of ^{125}I-labeled human IGF-I (Amersham Pharmacia Biotech, 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). Both IGFBP-2 and IGFBP-3 were quantified as digital light units per mm^2 and expressed in arbitrary units as a percentage of IGFBP-3 standard included on each gel.

Statistical Analyses

Longitudinal measurements of serum GH, IGF-I, and IGFBP-2 and IGFBP-3, morphometric data, body composition, and food intake were analyzed as repeated measures in a subplot, using a model that accounts for repeated samples from the same experimental unit (Gill and Hafs, ’71), with the mixed model analysis of variance procedure (SAS Inst. Inc., Cary, NC). The statistical model initially included type of fish fed as an independent variable; however, type of fish fed (herring or pollock) in the restriction phase (Phase II) was found to have no significant effect on the variables measured ($P > 0.05$) except percentage of body fat (reported in Jeanniard du Dot et al., 2008), and was therefore removed from the model. The final model included week of experiment, season experiment was performed, age of individuals, and all interactions. Values are presented as mean ± standard error (SE) unless otherwise noted. To evaluate differences among experimental weeks a pair-wise comparison of the Least Significant Difference was used. Differences were considered significant if $P<0.05$.

RESULTS

Intake and Morphometrics

Gross energy intake, body mass, and body composition, were previously reported (Jeanniard du Dot et al., 2008), and are briefly presented here to assess the response of the somatotropic axis in terms of intake and changes in body mass and composition of animals. The proximate composition of herring fed to the sea lions was 16% protein, 12% lipid and 68% water, whereas the composition of the pollock was 15% protein, 6% lipid, and 75% water. Caloric intakes were adjusted based on the body mass of the animal at the beginning of each experiment. At the start of the winter experiment animals weighed more than at the onset of the summer experiment (season $P<0.001$; 129.5 ± 1.2 kg summer, 142.6 ± 1.2 kg winter, Fig. 1A), so their intake was greater (season $P<0.001$; Phase I and III, 40.6 ± 1.6 kJ d^{-1} summer, 49.6 ± 1.6 kJ d^{-1} winter, Table 1).

As expected, animals lost approximately 10% of their body mass in the restriction phase (week $P<0.001$, Fig. 1B). Younger, smaller animals lost body mass at a faster rate compared with older, larger animals evaluated as a percentage of initial body mass (week × age $P=0.01$, Fig. 2A, B). There was a significant seasonal difference in subsequent patterns of body mass change (week × season $P<0.001$, Fig. 1A, B). In summer, animals failed to recover body mass in Phase III, controlled re-feeding. Substantial body mass gain was not observed until animals were fed ad libitum in Phase IV. In contrast, individuals gained body mass in the winter experiment during Phase III, and were only 2% below their starting body mass by the end of the controlled re-feeding diet.

Animals lost mostly adipose mass during the restriction diet (week $P<0.001$; Table 1). Overall, animals were leaner in summer compared with winter ($P<0.001$, Table 1); however, changes in adipose mass during the experiment varied by season (week × season $P=0.02$). Lean tissue mass declined during restriction (week $P<0.001$) and was greater in the winter relative to summer (season $P<0.001$), but the pattern of change in lean tissue was similar between seasons (week × season $P=0.15$; Table 1). On an absolute basis, both lean and adipose tissue were greater in sub-adults (both age $P<0.001$; Table 2); however, the percentage of adipose or lean tissue was similar ($P=0.83$ and $P=0.88$, respectively). Maintenance of lean tissue mass was different in juveniles and sub-adults and varied by season (age × season $P=0.02$; Table 2).

Standard length of individuals was stable within each season of the experiment (week × season $P=0.58$), but sea lions grew linearly between the end of the summer experiment and the beginning of the winter experiment (season $P<0.001$) when animals were fed ad libitum. Standard length increased an average of 7.6 ± 0.9 cm over the 25 weeks between experiments (189.0 to 196.6 ± 1.8 cm). Between the summer and winter experiments juveniles (8.2 ± 0.8 cm increase) had greater linear growth compared with sub-adults (4.9 ± 1.1 cm increase; age × season $P=0.004$).

Somatotropic Axis Components

Concentrations of IGF-I declined over Phase II (restriction) and remained low during Phase III (controlled re-feeding; week $P<0.001$, Fig. 1C). Overall IGF-I concentrations in winter were less than in summer (season $P<0.001$, Fig. 1C) and there was a significant interaction of week and season ($P<0.001$). Juvenile animals exhibited a greater decline in IGF-I concentrations in the restriction Phase II compared with sub-adults (week × age $P=0.02$, Fig. 2B, G). Concentrations of IGF-I increased in juveniles after 6 weeks of ad libitum feeding in the summer ($P=0.04$). Comparable blood samples from the ad libitum feeding Phase IV were not collected for sub-adults. The winter samples were collected after only 2 weeks of ad libitum feeding, therefore comparisons between seasons for Phase IV could not be conducted. There was a significant week–age–season interaction ($P=0.03$) indicating that juveniles and sub-adults had a differential response to restriction and re-feeding that was also subject to seasonal variation.

No statistical differences were observed in the overall GH concentration when compared by week ($P=0.35$); however, significant interactive effects were observed between week of experiment and season ($P=0.02$, Fig. 1E). In the summer, GH concentrations were less than winter concentrations from baseline measurements until the start of Phase III (controlled re-feeding) when summer GH concentrations increased and winter concentrations declined.

The concentrations of GH responded differently in juvenile and sub-adults to both nutrient restriction and re-alimentation (week × age $P=0.01$, Fig. 2C, H). Concentrations of GH in juveniles increased early in Phase II and again in early Phase III. The GH concentrations in sub-adults were more consistent across each phase of the experiment.

Concentrations of IGFBP-3 were stable until an increase was observed early in the controlled re-feeding (Phase III; $P=0.003$). There was a significant difference in the IGFBP-3 response between summer and winter (week × season $P<0.001$, Fig. 1D). Concentrations of IGFBP-3 declined slightly in summer, whereas winter concentrations spiked at the onset of Phase III. Concentrations of IGFBP-3 in juveniles and sub-adults varied by season (age × season $P=0.003$, Fig. 2E, J).

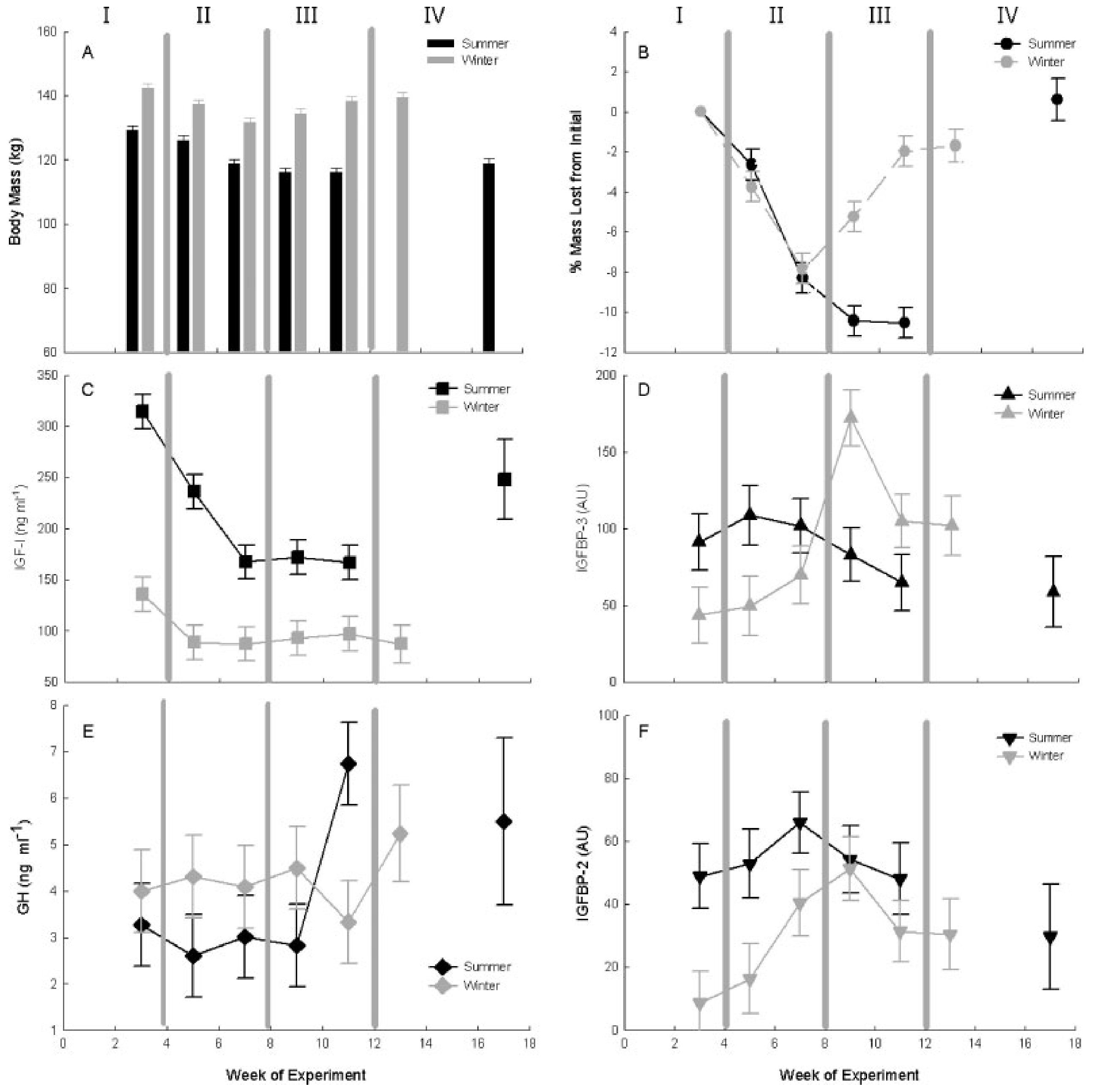

Figure 1. Seasonal differences in body mass and components of the somatotropic axis in Steller sea lions fed ad libitum (Phase I), followed by a restriction diet (Phase II), controlled re-feeding (Phase III), and ad libitum (Phase IV). Gray bars delineate phases of the experiment identified with roman numerals above graph. Symbols for the summer, long-day photoperiod, are black and the winter, short-day photoperiod, illustrated with gray symbols. Values for morphometrics including (A) total body mass and (B) percentage of mass lost from initial body mass, and concentrations of (C) insulin-like growth factor (IGF)-I, (D) insulin-like growth factor-binding protein (IGFBP)-3, (E) growth hormone (GH), and (F) IGFBP-2 are presented as mean ± SE. Week 13 values are from the winter experiment only. Week 17 data points represent values from juveniles in the summer experiment only.

Table 1. Seasonal difference in mean intake, body mass, and body condition for Steller sea lions fed ad libitum, restriction, and controlled re-feeding diets.[1,2,3]

		Intake (kJ d^{-1})		Body mass (kg)		Lean mass (kg)		Adipose mass (kg)	
Experiment phase	Week of experiment	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Ad libitum	3	40.6^{a}	49.6^{a}	129.5^{a}	142.6^{a}	105.8^{a}	114.7^{a}	22.3^{a}	26.2^{a}
Restriction	5	29.7^{b}	34.3^{b}	126.4^{b}	137.6^{b,e}	107.4^{a}	111.4^{a,b}	17.9^{b}	24.2
	7	31.9^{b}	34.3^{b}	119.0^{c}	131.9^{c}	103.0^{a}	109.2^{c}	14.9^{b}	21.3^{b}
Controlled re-feeding	9	40.4^{a}	50.0^{a}	116.3^{d}	134.7^{d}	97.5^{b}	106.4^{c}	17.7^{b}	27.1^{a}
	11	40.6^{a}	47.0^{a}	116.2^{d}	138.6^{b,e}	98.1^{b}	108.3^{c}	17.0^{b}	28.6^{a}
Ad libitum[4,5]	13	–	43.6^{a}	–	139.8^{e}	–	110.6^{b,c}	–	27.5^{a}
	17	43.2	–	89.8	–	75.3	–	19.9	–

[a–e] Different letters within a column indicate statistical difference observed between weeks of experiment ($P<0.05$).

[1] Within variable (intake, body mass, lean mass, or adipose mass) means were different by season ($P<0.001$).

[2] Seasons were designated as Summer for long-day photoperiod, and Winter for short-day photoperiod.

[3] Standard error was ±15.2 kJ d^{-1} for intake, ±1.2 kg for body mass, ±1.6 kg for lean tissue mass, and ±1.5 kg adipose tissue mass.

[4] Week 13 data collected in the winter trial only.

[5] Week 17 not included in pair-wise comparison because data were collected in the summer trial from juvenile animals only.

Concentrations of IGFBP-2 (week $P=0.02$) increased during feed restriction (Phase II), and declined during the controlled re-feeding (Phase III). Concentrations of IGFBP-2 in the summer were greater than winter concentrations (season $P=0.003$) with a significant interaction between week and season ($P=0.05$, Fig. 1F). Juveniles had greater concentrations of IGFBP-2 (age $P=0.03$) and the response of the two age categories were seasonally dependent ($P<0.001$, Fig. 2D, I). Both age classes had a similar IGFBP-2 response to restriction and controlled re-feeding. In contrast, in the summer juveniles maintained elevated concentrations of IGFBP-2 throughout the controlled re-feeding (Phase III), whereas concentrations in sub-adults declined.

DISCUSSION

The predictable response of the somatotropic axis to changes in nutrient intake and its key role in tissue-specific nutrient allocation make it a useful metric to evaluate nutritional status (Gautsch et al., '98; Harrell et al., '99; Connor et al., 2000; Freake et al., 2001; Rausch et al., 2002). Observations of components of the somatotropic axis in response to natural variation in nutrient intake in pinnipeds suggest that these species may respond in a similar manner to domestic mammals (Ortiz et al., 2003; Eisert et al. 2005; Richmond et al., 2010). Specifically, northern elephant seal and harbor seal pups exhibit increased GH and decreased IGF-I concentrations while fasting that are associated with increased utilization of adipose stores to meet energy needs (Ortiz et al., 2003; Richmond et al., 2010). Increased nutrient intake stimulates an increase in IGF-I concentrations in both adult female Weddell seals and harbor seal pups and is associated with accumulation of lean and adipose tissue (Eisert et al. 2005; Richmond et al., 2008, 2010). However, the nature of the physiological response to nutritional stress is not constant, and depends at least partly on concurrent life history demands. This study was conducted to investigate the effects of season and age on the response of the somatotropic axis to reduced nutrient intake and re-alimentation in Steller sea lions.

Response of the Somatotropic Axis to Restriction and Re-alimentation

As anticipated, concentrations of IGF-I initially parallelled changes in food intake and associated changes in body mass. During feed restriction (Phase II), animals lost approximately 10% of their body mass, and IGF-I concentrations were reduced 50%. However, the pattern was less consistent during recovery phases. In the summer, the decline in body mass and IGF-I stabilized when animals began controlled re-feeding (Phase III). However, the recovery of IGF-I and body mass were slower than anticipated; even after 3 weeks of controlled re-feeding, body mass and IGF-I concentrations remained similar to the restricted feed period (Phase II). After animals were fed ad libitum for 6 weeks, concentrations of IGF-I eventually increased, but were still less than concentrations before the start of the nutrient restriction. In the winter, the animals recovered most of their body mass during the controlled recovery period, but there was no parallel increase in IGF-I during this period of growth. However, increased concentrations of IGFBP-3 were observed during the winter in Phase III. This suggests that increased IGFBP-3 is required for a positive growth response to be observed during re-alimentation. Similar observations have been documented in cattle (Govoni et al., 2003; Velayudhan et al., 2007).

Significant increase in IGF-I concentrations are observed in other carnivore species within 2 weeks of re-feeding following

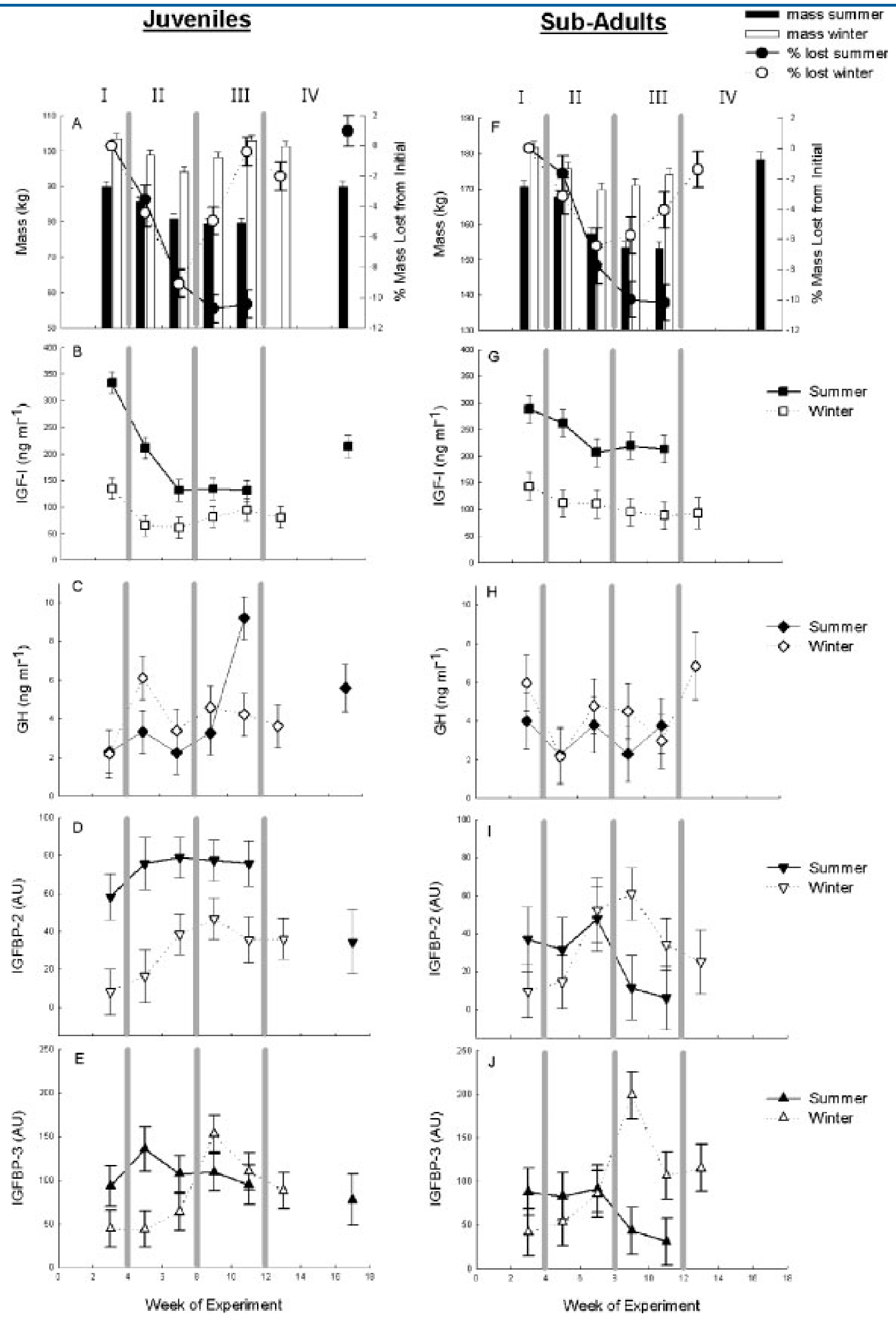

Figure 2. Seasonal response of juvenile and sub-adult Steller sea lions to ad libitum (Phase I), restriction (Phase II), controlled re-feeding (Phase III), and ad libitum feed (Phase IV) varies by age. Gray bars delineate phases of the experiment. Juvenile graph panels **(A–E)** are in the left column and sub-adult graph panels **(F–J)** are shown in the column to the right. Summer values are solid symbols and winter symbols are open. Values shown are mean ± SE. Panel (A, F) include body mass and percentage of mass lost relative to initial body mass. The response of metabolic hormones includes (B, G) insulin-like growth factor (IGF)-I, (C, H) growth hormone (GH), (D, I) insulin-like growth factor-binding protein (IGFBP)-2, and (E, J) IGFBP-3 concentrations. Week 13 data points represent values for the winter experiment only. Data shown in week 17 represent values from juveniles in the summer experiment only.

Table 2. Seasonal variation in body condition of juvenile and sub-adult Steller sea lions fed ad libitum, restriction, and controlled re-feeding diets.[1,2]

		Lean mass (kg)[3]				Adipose mass (kg)[4]			
		Summer		Winter		Summer		Winter	
Experiment phase	Week of experiment	Juvenile	Sub-adult	Juvenile	Sub-adult	Juvenile	Sub-adult	Juvenile	Sub-adult
Ad libitum	3	73.0^{a}	138.5^{a,b}	83.3^{a}	146.0^{a}	16.2^{a}	32.5^{a}	20.4^{a}	35.9^{a}
Restriction	5	73.9^{a}	140.9^{a}	81.0	141.7	12.1	27.4	18.1	34.4
	7	71.1	134.9^{b}	78.4^{b}	140.0^{b}	10.1^{b}	23.0^{b}	15.9^{b}	30.2^{b}
Controlled Re-feeding	9	64.8^{b}	130.2^{b,c}	75.7^{b}	137.0^{b}	14.4	23.9^{b}	22.7^{a}	34.4
	11	67.9^{b}	128.3^{c}	80.4	136.1^{b}	11.9^{b}	25.4^{b}	22.7^{a}	38.3^{a}
Ad Libitum[5,6]	13	–	–	79.8	141.4	–	–	21.7^{a}	37.5^{a}
	17	79.7	–	–	–	12.0	–	–	–

[a–c] Different letters within a column indicate statistical difference observed between weeks of experiment within age and season ($P<0.05$).

[1] Within tissue (lean or adipose) means were different by age and season (both $P<0.001$).

[2] Seasons were designated as Summer for long-day photoperiod, and Winter for short-day photoperiod.

[3] Standard error for lean tissue mass was ±1.9 in juveniles and ±2.5 sub-adults.

[4] Standard error for adipose tissue mass was ±1.8 in juveniles and ±2.3 sub-adults.

[5] Week 13 data collected in the winter trial only.

[6] Week 17 not included in pair-wise comparison because data was collected in the summer trial from juvenile animals only.

fasting (Maxwell et al., '99; Richmond et al., 2010). The failure of IGF-I to recover in this study may indicate that controlled re-feeding was not sufficient to meet energy requirements after an extended restriction diet, especially in the summer (Straus, '94; Smith et al., '95). This is further supported by changes in body condition where animals continued to lose lean tissue mass during the controlled re-feeding Phase III, and may indicate that IGF-I is predictive of adequate energy intake and energy balance.

In tandem with the decline in IGF-I concentrations during restriction, IGFBP-2 increased whereas IGFBP-3 remained consistent. In other mammalian species concentrations of IGFBP-3 decline with decreased nutrient intake, whereas IGFBP-2 concentrations increased (Gautsch et al., '98; Rausch et al., 2002). Circulating IGFBP-2 generally act to inhibit IGF-I interaction with target tissue receptors reducing the biological actions of IGF-I (Jones and Clemmons, '95). Increased concentrations of IGFBP-2 in sea lions would further limit the biological action of the already reduced circulating concentrations of IGF-I during the restriction phase. During the controlled re-feeding IGFBP-2 concentrations declined alleviating the inhibitory effect on target tissues.

The pattern of change in GH with restriction and re-alimentation was unexpected. The primary role of GH is to preserve body protein, especially in times of energy deficit (Johnsson and Hart, '86). Concentrations of GH generally increase with restricted nutrient intake and decrease with re-alimentation; however, similar to this study several studies have found variable results for GH response to nutrient restriction (Oster et al., '95; Hornick et al., 2000; Rausch et al., 2002; Ortiz et al., 2003; Fuglei et al., 2004; Nieminen et al., 2004). In this study, GH concentrations were stable throughout restriction (Phase II). The lack of overall GH response may be a result of the diel variability of GH concentrations or a reflection of the severity of the restriction.

Even though serum samples were taken at the same time of day and relative to the last meal consumed concentrations of GH were more variable than IGF-I. The variation in GH concentrations may have been due to the pulsatile nature of GH secretion (Zinn et al., '94; Tuggle and Trenkle, '96). Alternately, although fasting will induce an increase in circulating GH within 48 hr in pigs, in moderately feed-restricted animals, as in this study, the GH response may not be observed for 2 or more weeks (Hornick et al., 2000; Renaville et al., 2000; Barb et al., 2001). In this study, GH concentrations increased following the period of restriction, during controlled re-feeding (Phase III). This spike in GH was primarily due to increased concentrations in juveniles during the summer. The combinations of increased GH and IGFBP-2 and reduced IGF-I and IGFBP-3 in the controlled re-feeding phase suggest continued nutritional deficit in juveniles in the summer even though intake was increased (Richmond et al., 2010).

In response to energy deficits, GH acts directly on adipose tissue inhibiting utilization of nutrients by adipose allowing stored energy to be mobilized and used for maintenance energy needs (Eigenmann et al., '85; Mikel et al., '93). Even though GH failed to increase during the restriction phase, the majority of mass lost was from lipid (Jeanniard du Dot et al., 2008), indicating protein sparing (Castellini and Rea, '92; Rea et al., 2007). The lipid loss and protein sparing may have been due to the increased cortisol concentrations in response to acute nutritional stress

(Jeanniard du Dot et al., 2009). Cortisol is an important stimulator of increased fatty acid metabolism and acts synergistically with GH to metabolize fat (Ortiz et al., 2001). In northern elephant seal pups, GH concentrations only increased from 3 to 4 ng ml^{-1}, even after 5–7 weeks of fasting; however, this minor increase in GH concentration was associated with utilization of adipose tissue stores (Ortiz et al., 2003). Concentrations of GH (4 ng ml^{-1}) were similar in this study. It is possible that the GH response is blunted in species that are adapted for fasting. Alternately, the combination of moderate GH concentrations and increased cortisol in conjunction with reduced IGF-I is sufficient to mobilize energy from adipose and conserve body protein.

During controlled re-feeding (Phase III) animals gained mostly fat while lean tissue declined. Accumulation of significant lean tissue did not occur until animals were fed ad libitum (Phase IV). Under adequate nutrition increased GH stimulates IGF-I and IGFBP-3 and inhibits IGFBP-2 production in liver and peripheral tissues (Le Roith et al., 2001) stimulating proliferation and differentiation of structural tissue, including muscle and bone (Mikel et al., '93; Govoni et al., 2005). The results in this study suggest a similar role of the somatotropic axis in regulating nutrient partitioning of Steller sea lions. Concentrations of GH remained low throughout restriction and most of re-alimentation. After 4 weeks of controlled re-feeding GH concentrations increased, after which animals began to accumulate lean tissue.

The pattern of increased concentrations of GH and IGF-I was observed in juveniles in the summer after 6 weeks of ad libitum feeding. This hormone response was associated with increased linear growth (indicative of skeletal growth) and increased total body mass, including significant accretion of both lean and adipose tissue. After 6 weeks of ad libitum feeding, juvenile sea lions gained 3 kg of adipose, 2 kg of lean tissue, and grew 2.5 cm in length. Before the start of the winter experiment, sea lions had gained an additional 6 kg of adipose, 2 kg of lean tissue, and 5.7 cm in length.

Seasonal Influence on the Response of the Somatotropic Axis

During the re-alimentation Phase III, a difference between seasonal replicates of the experiment was observed. Although animals in the summer failed to recover body mass in the controlled re-feeding Phase III, during the winter experiment, individuals increased their body mass, and by the end of Phase III attained a mass similar to the experiment start. This seasonal difference in recovery of body mass may suggest that animals are better able to recover from a nutritional deficit in winter months, or that there is a seasonal signal that regulates energy allocation.

The seasonal difference in IGF concentrations was striking. Concentrations in the summer were twice those in winter. Difference in IGF-I concentration may reflect seasonal differences in energy partitioning, where animals minimize protein accretion in short-day photoperiods (Zinn et al., '86; Webster et al., '99). Many species have differential seasonal rates and composition of gain. In mice and cattle, long photoperiod induces lean tissue accretion, whereas short photoperiod induces increased fat accretion (Zinn et al., '86; Genin and Perret, 2000). As a primary role of IGF-I is to stimulate protein accretion, decreased concentrations would minimize lean tissue accretion (Gautsch et al., '98; Hornick et al., 2000), whereas increased concentrations of GH would inhibit nutrient utilization by adipose (Eigenmann et al., '85; Johnsson and Hart, '86). This is supported by the observation in this study that animals were leaner in summer and fatter in winter. Animals in the summer were also better able to spare lean tissue in the restriction and controlled re-feeding phases (II and III, respectively). Even though composition of the animals was similar at the start of the summer and winter experiments, animals lost proportionally less fat in winter compared with summer, although on an absolute basis, animals lost 3 kg of adipose regardless of season.

Concentrations of GH were generally greater in winter compared with summer, similar to observations in other carnivore species (Fuglei et al., 2004). However, the pattern of response was not similar between seasons. Contrary to the rapid increase in GH concentration in the summer controlled re-feeding (Phase III), GH concentrations declined in winter Phase III. This decline was associated with a large increase in fat mass (7 kg) that was not observed in the sea lions during the summer controlled re-feeding (Phase III, 2 kg gain). Some mammalian species have increased GH in winter months, likely due to increased melatonin, independent of energy restriction (Zinn et al., '88; Nieminen et al., 2002). Steller sea lions also appear to have strong seasonal changes in body composition and composition of mass gain that may be signaled by photoperiod and regulated by the somatotropic axis.

Interestingly, IGFBP-3 spiked during the winter restriction Phase III, whereas concentrations declined in the summer. The increase in IGFBP-3 in winter was associated with increased growth rate, in contrast to the summer trials when animals did not gain body weight. Circulating IGFBP-3 functions to extend the half-life of IGF-I and facilitate interactions with target tissue receptors (Jones and Clemmons, '95). Increased concentrations of IGFBP-3 are generally associated with an increased growth rate (Skaar et al., '94; Harrell et al., '99; Rausch et al., 2002; Govoni et al., 2003). Even though IGF-I concentrations remained low in winter, the spike in IGFBP-3 could account for the increased growth observed in the winter, whereas the absence of increased IGFBP-3 in summer could account for the lack of a growth response in that season. Concentrations of IGFBP-2 were much less in winter than summer. IGFBP-3 is generally positively associated with growth, whereas IGFBP-2 is negatively associated with growth. The reduced concentrations of IGFBP-2 may have further facilitated the recovery of body mass of animals in winter by reducing the inhibitory influence of IGFBP-2 on IGF-I action. In agreement with Steller sea lions in this study, research in rats and cattle have shown that IGFBP-3 was a better predictor of growth rate than IGF-I (Mandel et al., '95; Govoni et al., 2004), and further that an increase in IGFBP-3 was required before gains in body mass were observed (Govoni et al., 2003; Velayudhan et al., 2007; Yamada and Lee, 2009).

Effect of Age on the Response of the Somatotropic Axis

Both juveniles and sub-adults lost body mass in this study; however, juveniles lost body mass at a faster rate compared with older animals. Sub-adult sea lions had greater overall concentrations of IGF-I compared with juveniles in summer and winter. In addition, although juveniles and sub-adults experienced a decline in IGF-I concentration at the onset of the restriction Phase II, the decline in IGF-I in juveniles was much greater. Further, GH concentrations in juveniles increased at the onset of nutrient restriction, whereas concentrations at the same time point in sub-adults declined. Research in multiple species has found that the transition from fed to fasting state occurs more rapidly in young animals relative to mature animals, likely due to the greater metabolic rate, smaller energy reserves, and greater growth requirements of young animals (Cameron et al., '85; Barb et al., '97; Rea et al., 2009). These data suggest that regardless of season older, larger animals are better able to cope with stress associated with energy deficit.

As expected, IGFBP-2 increased in response to nutrient restriction and decreased during re-alimentation. However, overall concentrations were greater in juvenile sea lions compared with sub-adults. In domestic animals, IGFBP-2 increases with age and is negatively associated with growth rate (Harrell et al., '99; Govoni et al., 2004). The increased concentration observed in juvenile sea lions may be a further indicator of increased sensitivity in young animals to restricted feeding. This may also indicate that the controlled re-feeding diet in Phase III in which young animals were not gaining body mass was actually a nutrient-restricted diet relative to their energy needs for growth.

In addition to the effect of dietary energy on the somatotropic axis, composition of the diet can also influence components of the somatotropic axis. For example, limitation of dietary protein is associated with reduced growth rate, IGF-I, and IGFBP-3, but increased IGFBP-2 (Kriel et al., '92). Thus, changes in both the quality and (or) quantity of feed can influence components of the somatotropic axis. Pollock has less energy density, less lipid content, and greater water content than herring. Previous research has shown that Steller sea lions fed restriction isocaloric diets lose more lipid mass when they are fed fish with less fat content (Rosen and Trites, 2005; Kumagai et al., 2006). This illustrates the importance of diet composition on energy maintenance. However, in this study we found no effect of type of fish fed on body composition or components of the somatotropic axis. Research investigating the effect of diet composition and season on body condition of ad libitum-fed sea lions found that seasonal changes in physiology have a greater impact on body condition than type of prey consumed (Rosen and Trites, 2005; Atkinson et al., 2008a). Results of this study similarly suggest that seasonal patterns are a more important factor for determining variation in body condition and metabolic hormones than composition of prey consumed. These results are significant when using captive animal research to interpret data from free-ranging individuals. As free-ranging individuals consume a diverse diet and precise composition of intake is often unknown, metabolic indicators, such as the somatotropic axis, that are insensitive to composition of intake but sensitive to energy intake may prove to be useful tools for evaluating nutritional status of free-ranging animals.

CONCLUSION

Since the early 1990s, considerable research has focused on the potential role of nutritional stress in the decline of the endangered Steller sea lion (Atkinson et al., 2008b). However, little is known about the mechanism by which decreased nutrient intake influences growth physiology of Steller sea lions. To determine whether nutritional stress was a cause or contribution to the decline, and subsequent population recovery failure, we must have a greater understanding of the physiological mechanisms that Steller sea lions use to cope with decreased nutrient intake, and how decreased intake influences growth and possibly survival of these marine mammals.

Based on the domestic animal model of the somatotropic axis response to nutrient restriction and re-alimentation, Steller sea lions shared some interesting similarities to domestic species; however, several components in the current experiment did not respond as predicted. Body mass, body fat, and IGF-I declined as predicted for restriction diet. The pattern of increased GH with reduced IGF-I and IGFBP during the controlled re-feeding phase suggests that animals did not reach positive energy balance until intake surpassed maintenance. Importantly, these data indicate that measurement of GH or IGF-I alone is not sufficient to determine nutritional status of individuals. Multiple components of the somatotropic axis must be measured to accurately assess nutritional status. Additionally, hormone concentrations appear to be insensitive to type of fish fed. This may be important when considering the use of these hormones as a metric of nutritional stress in wild populations. Animals in the wild eat a mixed diet, and at the time of sampling previous diet will likely be unknown. Developing metrics of nutritional status that are sensitive to energy intake, but insensitive to the composition of intake will prove useful in evaluating nutritional status of populations of marine mammals.

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