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Changes in glucocorticoids, IGF-I and thyroid hormones as indicators of nutritional stress and subsequent refeeding in Steller sea lions (*Eumetopias jubatus*)

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ABSTRACT

Physiological responses to changes in energy balance are tightly regulated by the endocrine system through glucocorticoids, IGF-I and thyroid hormones. Changes in these hormones were studied in eight captive female Steller sea lions that experienced changes in food intake, body mass, body composition, and blood metabolites during summer and winter. During a period of energy restriction, one group of sea lions was fed reduced amounts of Pacific herring and another was fed an isocaloric diet of walleye pollock, after which both groups returned to their pre-experimental diets of herring. Cortisol was negatively and IGF-I was positively associated with changes in body mass during periods of energy restriction (mass loss associated with increase in cortisol and decrease in IGF-I) and refeeding (body mass maintenance associated with stable hormone concentrations in summer and compensatory growth linked to decrease in cortisol and increase in IGF-I in winter). Cortisol and IGF-I were also correlated with changes in lipid and lean mass, respectively. Consequently, these two hormones likely make adequate biomarkers for nutritional stress in sea lions, and when combined provide indication of the energetic strategy (lipid vs lean mass catabolism) animals adopt to cope with changes in nutrient intake. Unlike type of diet fed to the sea lions, age of the animals also impacted hormonal responses, with younger animals showing more intense hormonal changes to nutritional stress. Thyroid hormones, however, were not linked to any physiological changes observed in this study.

1. Introduction

Natural habitats are neither constant nor completely predictable, and animals must continuously adjust to these environmental variations (Wingfield and Kitaysky, 2002). Regulation of energy intake and expenditure is an essential part of these adjustments, and is linked to the survival and reproductive success of animals in their natural environment (Lavigne et al., 1982). Insufficient quantity or quality of food, conditions that Steller sea lions might chronically encounter in the wild (Trites and Donnelly, 2003), results in animals modifying their physiologies and behaviours to rebalance their energy budgets (Stini, 1969; Waterlow, 1986; Lawson et al., 1997; Schmidt-Nielsen, 1997; Boyd, 2002; Trumble et al., 2003). Ultimately these physiological changes are regulated by complex, interconnected endocrine systems. Determining how changes in hormone concentrations are influenced by external factors such as diet type, age, and season that all impact nutritional physiology of Steller sea lions during periods of energy restriction (Rosen and Trites, 2005; Kumagai et al., 2006; Jeanniard du Dot et al., 2008), can help to understand the regulatory mechanisms behind changes in body composition, partitioning of the energy intake, and ultimately life history of these animals.

Endocrine response to nutritional stress (in terms of food quantity or quality) primarily, but not exclusively, involves the somatotropic, glucocorticoid, and thyroid hormones (Hornick et al., 2000; Robson et al., 2002). Hormones from the somatotropic axis, including insulin-like growth factor-I [IGF-I], are involved in protein and lipid metabolism as well as mineral metabolism and bone growth (Breier, 1999; Butler and Le Roith, 2001) while glucocorticoids (cortisol and free cortisol) are known to be involved in lipid metabolism, and correlate with unpredictable life situations such as energy intake shortages (Kitaysky et al., 2001b; Reeder and Kramer, 2005). Thyroid hormones (triiodothyronine [T3], and thyroxine [T4]) are essential for growth, metabolism, and thermogenesis in mammals (Kelly, 2000). During a negative energy balance, GH and glucocorticoids usually increase while concentrations of IGF-I and thyroid hormones decrease (Table 1) which are linked to a cessation of growth, a sparing of protein and an increase in lipolysis (Hornick et al., 2000), an overall decrease in energy expenditures through reduction in metabolic rates (Buonomo and Baile, 1991; Yambayamba et al., 1996; Weinsier et al., 2000), and a modification of development and behaviour in birds and mammals (Moberg, 1985; Ortiz et al., 2001a; Kitaysky et al., 2003).

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Table 1
Predicted and observed changes of blood concentrations of cortisol and free cortisol, IGF-I, total T3, total and free T4, BUN/creatinine, NEFA and β-HBA in 8 female Steller sea lions during period of energy restriction followed by periods of refeeding in summer and winter

Parameter	Predicted results[a]		Observed results				Related to loss of endogenous energy reserves
	Energy restriction	Refeeding	Energy restriction		Refeeding		
			Summer	Winter	Summer	Winter	
Cortisol	↑	↓	↑	↑[b]	0	↓[b]	− (lipids)
Free cortisol	↑	↓	↑	↑[b]	0	↓[b]	− (lipids)
IGF-I	↓	↑	↓[b]	↓	0	0 or ↑[b]	+ (proteins)
Total T3 (TT3)	↓	↑	↓	↓ or ↑[c]	0	↑	NA
Total T4 (TT4)	↓	↑	0	↓	0	↑	NA
Free T4 (fT4)	↓	↑	0	0	0	0	NA
TT3:TT4	↓	↑	↓[c]	↓ then ↑[c]	↓	0	NA
BUN:creatinine	↑	↓	0	↑ or 0[c]	↑	↓ or 0[c]	− (proteins)
NEFA	↑	↓	↓[c]	↑	0	↓	no
β-HBA	↑	↓	0	NA	0	NA	no

Significant relations between changes in blood variables and changes in endogenous energy reserves during the course of the experiments are also indicated.
− and + means negatively and positively related to endogenous energy reserves (type in brackets).
[a] Predicted results are based on literature information from (Bergendahl et al., 1996; Buonomo and Baile, 1991; Castellini et al., 1993; Eales, 1988; Hornick et al., 2000; Kitaysky et al., 2001a,b; Moberg, 1985; Rea et al., 1998; Reeder and Kramer, 2005; Renaville et al., 2002; Robson et al., 2002; Thissen et al., 1994; Weinsier et al., 2000).
[b] means difference between age groups.
[c] means difference between diet groups.

While somatotropic, glucocorticoid, and thyroid hormones regulate lipid or protein metabolism during periods of energy restriction, changes in tissue catabolism should also be reflected in blood concentrations of relevant metabolite by-products. In fasting animals, concentrations of non-esterified fatty acids (NEFA) and ketone bodies (beta-hydroxybutyrate, [β-HBA]) usually indicate lipid catabolism while concentrations of nitrogen waste in the blood (blood urea nitrogen, BUN) reflect changes in protein catabolism (Castellini et al., 1993; Rea et al., 2000; Mellish and Iverson, 2001). However, these variables may not accurately reflect the source of tissue catabolism during a milder energy restriction (when the rate of mass loss is less than during a fast) and will be additionally affected by concentrations of dietary protein and lipid intake (Narayanan and Appleton, 1980; Ramsay et al., 1991).

Interpreting the relationship between hormones and responses to nutritional stress is complex. Metabolism and hormone concentrations of mammals in general, and Steller sea lions, in particular, may vary naturally throughout the year with photoperiod or breeding status, and by age of the animals independent of nutritional stress (Engelhardt and Ferguson, 1980; Little, 1991; Thissen et al., 1994; Hall et al., 1998; Haulena et al., 1998; Wingfield and Kitaysky, 2002; Kumagai, 2004b; Myers et al., 2006; Mashburn and Atkinson, 2007). Concentrations of glucocorticoids are generally greater during the breeding season in summer in vertebrate species (Sangalang and Freeman, 1976; Bartsh et al., 1992; Romero, 2002), while circulatory concentrations of IGF-I are greater in younger animals (Aujard et al., 2004) and in humans (Lamberts et al., 1997). Type of diet (protein/lipid ratio) also seems to influence hormone concentrations (Nap et al., 1993) and physiological response to nutritional stress (Kumagai et al., 2006; Jeanniard du Dot et al., 2008). Consequently, it is essential to use controlled studies to separate the impact of each variable (diet quality, level of energy intake, season, and age of the animal) on the endocrine systems to understand the potential impacts of resulting physiological responses during periods of energy restriction.

The primary goal of this study was to investigate the impact of season, age, and type of diet on the endocrine regulation in Steller sea lions during and after nutritional stress. Hormone measurements were discussed in reference to changes in body mass, body composition, and energetic priorities. We hypothesized that the endocrine response to nutritional stress would differ between animals fed a different type of diet since it induces differential loss of lean and lipid mass (Kumagai et al., 2006; Jeanniard du Dot et al., 2008). We also expected the endocrine response to nutritional stress would be more intense in winter when energetic demands are greater, and in younger animals that have greater mass-specific energetic needs compared with older sea lions (Winship et al., 2002). Efficacy of blood metabolites and hormones as tools to evaluate nutritional stress of the animals was also assessed. Defining the relationship between the endocrine system and metabolic and energetic status will expand our understanding of the mechanisms of energetic adjustments available to Steller sea lions, and ultimately shed light on how changes in diet quality and quantity may be affecting animals' health in the wild.

2. Material and methods

2.1. Experimental design

Our study was conducted with the approval of the University of British Columbia Animal Care Committee, under Permit No. A04-0169. Experiments were conducted at the Vancouver Aquarium (BC, Canada) in the summer (June–August) 2005 and winter (January–March) 2006, on 8 female Steller sea lions (*Eumetopias jubatus*) (five 3-year old juveniles and three 5-year old sub-adults). The animals were randomly assigned to one of the two experimental groups, Group H or Group P, which were kept identical for the two seasons.

Each trial consisted of three phases, starting with a 28-day baseline (B) during which all the animals were fed their usual daily ration (i.e. energy intake to maintain body mass or sustain a slow growth) of Pacific herring (*Clupea pallasii*). The sea lions were then placed on a 28-day restriction treatment (R) during which energy intake was reduced by approximately 20–30%. Juveniles in both diet groups were given 260 kJ kg^{-1} d^{-1} of food and sub-adults received 230 kJ kg^{-1} d^{-1} (to buffer age/size differences and to result in a maximum 15% change in body mass). The two diet groups were given the same restricted level of energy (isocaloric diets), but Group H was fed exclusively Pacific herring and Group P was fed only walleye pollock (*Theragra chalcogramma*). Lipid and energy contents were less for pollock than for herring which resulted in Group P eating 30% and 60% more fish than Group H during the summer and winter energy restrictions, respectively, in order to obtain similar energy intakes between the two groups. The restriction was followed by a 28-day controlled refeeding (CR) period during which each sea lion received the same diet and energy intake of Pacific herring as they had received during the baseline phase. Subsamples of the herring and pollock were analyzed for their proximate chemical composition by Northwest Labs (Surrey, BC, Canada) and the gross energy contents of the

fish were calculated using the energy conversion factors provided by Schmidt-Nielsen (1997): 39.3 kJ g^{-1} for lipid and 18 kJ g^{-1} for protein (proximate composition of fish available in, Jeanniard du Dot et al., 2008). No vitamins or medicines were added to the diet during the experiments.

2.2. Data acquisition

Food intake (±0.02 kg d^{-1}), energy intake (kJ d^{-1}), and body mass of each animal (±0.1 kg, animal voluntary standing still on a scale) were recorded daily. Measurements of body composition and blood sample collection were performed in the morning before the first meal of the day (~18 h overnight fast) and every two weeks (at the end of the baseline period [B4], during 2nd and 4th week of restriction [R2, R4], and of controlled recovery [CR2, CR4]). Blood samples were taken from the caudal gluteal vein at similar times in the day (±1 h) throughout the experiments to avoid potential influence of circadian rhythms on circulating hormone concentrations. Procedures were performed while animals were under anaesthesia (isoflurane) and with veterinary supervision. Blood samples for hormone concentration and isotopic measurements were collected into serum separator tubes and EDTA tubes (plasma) and were centrifuged (959 *g* for 5 min). Sera and plasma were then stored at −70 °C. Body composition was determined using the deuterium dilution technique validated in pinnipeds (Reilly and Fedak, 1990). Total body water, body fat mass and fat-free or lean mass were calculated using the equation derived from Arnould et al. (1996) (more details on the procedure in Jeanniard du Dot et al., 2008). Changes in the percent of fat and lean mass over time and changes in either component expressed as a percent of the total body mass loss were calculated in reference to the baseline measurement.

Thyroid hormones (total thyroxine [TT4], total triiodothyronine [TT3] and free thyroxine [fT4]) and blood metabolites (BUN, creatinine, NEFA and β-HBA) concentrations were measured at the Central Veterinary Laboratory (Langley, BC, Canada). Thyroid hormones were measured by radioimmunoassay (RIA). Total T4 was measured using Immulite® canine Total T4 kit (Siemens Medical Solution Diagnostics) with intra-and inter-assays variation of 3.9% and 5.2%. Total T3 was measured using Coat-A-Count® Total T3 kit (Siemens Medical Solution Diagnostics) that had 6.6% and 5.9% of intra- and inter-assay coefficient of variations. Concentrations of BUN and creatinine were measured using Urea Nitrogen Flex™ and Creatinine Flex™ reagent cartridges (Dimension® clinical chemistry system). Intra- and inter-assay coefficients of variation were 2.6% and 4.8% respectively for the BUN tests and 0.6% and 1.1% for the creatinine tests. The BUN:creatinine ratio was used as an indicator of protein catabolism (BUN) while controlling for the impact of protein load in the diet on the BUN concentrations (creatinine). Non-esterified fatty acids and β-HBA were measured by enzymatic colorimetric methods using the Wako NEFA C test kit (ACS-ACOD method) and the enzymatic β-hydroxybutyrate reagent set (Pointe Scientific, Iic.). Intra- and inter-assay variations were 1.1% and 2.7% for NEFA respectively and 1.7% and 5.2% for β-HBA.

Concentrations of IGF-I were quantified by RIA previously validated for Steller sea lion serum at the University of Connecticut (Richmond, 2008) using rabbit-anti-human antisera as the primary antibody (AFP4892898, National Hormone & Peptide Program), and the secondary antisera to rabbit γ-globulin was produced in goats (Calbiochem EMB Biosciences). Intra-assay variation was 5.1% and inter-assay variation was 6.4%. Glucocorticoids were analysed at the University of Alaska Fairbanks using techniques validated for Steller sea lions detailed in Kitaysky et al. (2007). Cortisol was analysed by RIA in duplicates using cortisol antiserum 07-121016 (ICN Biomedical Inc. Costa Mesa CA, USA). The sensitivity of the technique was 7.8 pg/tube and the intra- and inter-assay variations were 2% and 9% respectively. Free cortisol was measured using a cortisol-binding globulin assay according to previously established protocols (Breuner and Orchinik, 2002; Love et al., 2004). Average intra- and inter-assay and inter-assay variations were 2.5% and 6.2% respectively. Free cortisol titers were estimated using the equation of Barsano and Baumann (1989).

Daily (DMR) and standard (SRM_A) metabolic rates in air were measured by open circuit respirometry every two weeks concurrent with body composition and hormone concentration determinations. All metabolic procedures are detailed in Jeanniard du Dot et al. (in press). Sea lions were enclosed in a large metabolic chamber containing a small pool with enough room for the animals to perform their daily routine during 24 h for measurement of DMR. They were enclosed in a small metabolic dry chamber for 1 h for measurement of SMR_A.

2.3. Statistical analyses

Repeated-measures variables in these experiments were analyzed with linear mixed-effects models (lme) using R 2.4.0 (nlme library from Pinheiro et al., 2007). The lme models were fitted such that comparisons within a variable over the course of the experiments

Table 2
Body mass, body condition, gross energy intake, and fish biomass intake of eight female Steller sea lions in diet groups H and P in summer 2005 and winter 2006

Season	Diet group	Phase	Mass (kg)	Mass change (%)	Body condition (% fat)	Body condition change (%)	Gross energy intake (kJ/d)	Fish biomass intake (kg/d)
Summer	H	B4	108.88 (21.18)	NA	20.10 (1.65)	NA	35.43 (5.75)	4.83 (0.78)
		R2	110.63 (23.24)	−4.53 (0.19)	14.83 (1.13)	−27.94 (6.43)	27.07 (3.92)	3.69 (0.53)
		R4	98.65 (18.97)	−9.29 (1.10)	12.23 (1.46)	−37.43 (10.32)	27.07 (3.92)	3.69 (0.53)
		CR2	101.37 (20.68)	−12.26 (0.83)	16.59 (0.66)	−17.92 (12.40)	34.65 (6.07)	4.83 (0.78)
		CR4	96.10 (17.12)	−11.06 (1.44)	17.06 (1.89)	−14.45 (7.95)	34.65 (6.07)	4.83 (0.78)
	P	B4	131.35 (23.59)	NA	16.96 (1.20)	NA	40.73 (7.13)	5.55 (0.97)
		R2	128.45 (23.60)	−2.45 (0.55)	15.28 (0.77)	−8.33 (9.09)	31.64 (4.64)	5.82 (0.85)
		R4	120.40 (21.81)	−8.42 (0.61)	14.22 (1.28)	−15.92 (5.99)	31.64 (4.64)	5.82 (0.85)
		CR2	118.10 (21.66)	−10.29 (0.69)	17.43 (1.89)	−13.47 (10.10)	39.78 (6.74)	5.55 (0.97)
		CR4	118.33 (21.76)	−10.14 (0.70)	14.26 (0.58)	−14.27 (8.48)	39.78 (6.74)	5.55 (0.97)
Winter	H	B4	121.70 (19.73)	NA	20.21 (0.86)	NA	47.59 (5.21)	6.13 (0.67)
		R2	115.45 (18.81)	−5.17 (0.22)	17.79 (1.14)	−13.56 (3.45)	30.34 (3.78)	3.91 (0.49)
		R4	110.50 (18.88)	−9.51 (0.85)	14.65 (1.44)	−26.98 (8.15)	30.34 (3.78)	3.91 (0.49)
		CR2	114.85 (18.10)	−5.46 (0.73)	22.14 (2.04)	18.20 (16.98)	45.33 (5.71)	5.84 (0.74)
		CR4	119.50 (17.74)	−1.27 (1.40)	22.34 (0.71)	10.69 (1.50)	45.33 (5.71)	5.84 (0.74)
	P	B4	143.60 (21.94)	NA	19.32 (1.43)	NA	47.53 (4.58)	6.13 (0.59)
		R2	139.85 (21.76)	−2.74 (0.65)	19.74 (1.66)	2.23 (4.08)	34.35 (3.94)	7.76 (0.89)
		R4	134.30 (21.19)	−6.68 (1.14)	19.63 (1.46)	1.65 (1.24)	34.35 (3.94)	7.76 (0.89)
		CR2	136.25 (20.65)	−5.04 (1.16)	21.74 (2.31)	11.86 (4.89)	46.74 (4.66)	6.02 (0.60)
		CR4	139.75 (20.14)	−2.26 (1.06)	21.76 (1.57)	13.82 (9.09)	46.74 (4.66)	6.02 (0.60)

Measurements were taken at the end of the baseline (4th week, B4) and at week 2 and 4 of the restriction (R2 and R4) and of the controlled refeeding (CR2 and CR4). The rates of change are calculated in reference to concentrations at the end of the baseline (B4). Numbers in brackets represent the standard errors of the means.

were made in reference to the baseline measurement before the start of the restriction. Models' assumptions were verified and any autocorrelation or heterogeneity of variance of within-group residuals was corrected for each model as explained by Pinheiro and Bates (2000). Fixed effects considered were diet group, experimental phase, age, season, or interaction between these terms. Random effects grouped by individuals depended on best models fit. Sample size for all hormones and blood metabolites was 40 in the summer and 47 in the winter. Relationships between concentrations of hormones or metabolites and body mass, body composition, and daily or standard metabolic rates (DMR or SMR_A) of the animals were also analyzed using linear mixed effect models. The best model fits were estimated by AIC. Data values provided as means ± SE and all p values were extracted from the mixed effect model summaries. Statistical significance for each parameter estimate was set at $\alpha = 0.05$.

3. Results

3.1. Body mass and body composition

The body mass and body composition data were detailed in Jeanniard du Dot (2008) and are summarized in Table 2. Animals lost approximately 10–15% of their initial body mass during the periods of energy restrictions. Rates of mass loss were similar in both seasons ($p > 0.17$) and between diet ($p > 0.55$) and age groups ($p > 0.06$). In summer, animals in Group H lost the majority of body mass from body fat and conserved lean tissues, while Group P lost significantly less body fat and more lean tissues (all $p < 0.01$), as determined by differences in body composition between the different phases. In winter, lipid loss was overall slightly (but not significantly, $p = 0.11$) less than in summer for all animals. Group H again lost significantly more body fat and less lean mass compared with Group P ($p < 0.01$) that lost around 80% of their body mass as lean mass. During the subsequent controlled refeeding in summer, the animals did not regain body mass when returned to their baseline diet but remained stable at the same mass as at the end of the restriction treatment ($p = 0.24$). During this month, proportion of lean and fat tissues in the body attained pre-restriction levels for both groups ($p > 0.46$). During the winter controlled recovery, however, all sea lions rapidly regained the previously lost mass due to rates of mass gain that were 7.3 ± 1.4% greater than the rates of mass gain observed during the baseline period ($p < 0.0001$). This compensatory mass gain was accomplished mostly by storing body fat. The age of animals had no significant effect on the changes observed in body composition at any time throughout the experiments or in any season (all $p > 0.05$).

3.2. Glucocorticoids

3.2.1. Effects of diet composition

Experimental diet (low-quantity high-energy fish for Group H and high-quantity, low-energy fish for Group P during the restrictions) was never a factor impacting absolute concentrations or relative changes in glucocorticoids during the experiments (all $p > 0.10$).

3.2.2. Effects of age and season

In summer, relative changes (compared with baseline concentrations) of cortisol and free cortisol concentrations were similar for both age groups during the restriction and the controlled refeeding ($p = 0.46$ and $p = 0.14$) even though juveniles started the experiments with greater baseline concentrations (cortisol: 93.70 ± 5.13 ng mL^{-1} and 68.58 ± 5.00 ng mL^{-1}, free cortisol: 27.12 ± 4.32 ng mL^{-1} and 6.00 ± 2.48 ng mL^{-1} for juveniles and subadults respectively, Table 3). Animals responded to the experimental restriction of daily energy intake by increasing glucocorticoid secretion (Fig. 1 and Table 1). Concentrations of cortisol were 20% greater and concentrations of free cortisol were approximately three times greater during both the restriction and the controlled refeeding, compared with the baseline concentrations, (all $p < 0.04$; Fig. 1a, b).

Table 3
Serum concentrations of total and free cortisol and IGF-1 of eight female Steller sea lions during the summer 2005 and winter 2006 experiments

Season	Phase	Age	Total cortisol (ng ml^{-1})	Free cortisol (ng ml^{-1})	IGF-1 (ng ml^{-1})
Summer	B4	Juvenile	93.70 (5.13)	27.12 (4.32)	333.39 (22.56)
	R2		102.61 (4.57)	52.18 (4.51)	211.18 (16.02)
	R4		104.17 (3.04)	49.68 (3.65)	131.30 (12.80)
	CR2		110.67 (4.66)	55.48 (5.22)	133.87 (23.63)
	CR4		109.56 (8.34)	47.00 (9.78)	130.75 (21.88)
	B4	Subadult	68.58 (5.00)	6.00 (2.48)	288.25 (69.04)
	R2		91.75 (15.05)	39.67 (10.90)	261.80 (52.84)
	R4		85.98 (0.83)	28.10 (0.40)	206.59 (19.04)
	CR2		80.11 (7.82)	16.98 (5.56)	219.07 (28.16)
	CR4		90.15 (10.53)	23.87 (8.94)	213.29 (19.57)
Winter	B4	Juvenile	82.91 (6.51)	12.91 (4.86)	134.67 (13.62)
	R2		103.32 (4.10)	34.51 (5.93)	65.46 (4.11)
	R4		114.91 (6.10)	51.75 (6.38)	61.10 (8.80)
	CR2		109.79 (7.29)	41.69 (5.75)	81.84 (13.39)
	CR4		102.26 (4.75)	33.77 (2.13)	94.22 (5.72)
	B4	Subadult	78.03 (6.37)	10.39 (4.67)	143.41 (23.97)
	R2		97.16 (7.75)	26.18 (2.03)	111.42 (17.75)
	R4		91.89 (6.51)	30.72 (12.12)	94.84 (21.41)
	CR2		102.59 (9.89)	32.14 (12.37)	109.79 (39.24)
	CR4		99.69 (9.50)	32.25 (15.38)	87.96 (20.55)

Measurements were taken at the end of the baseline (4th week, B4) and at week 2 and 4 of the restriction (R2 and R4) and of the controlled re-feeding (CR2 and CR4). The averages are given per age group and numbers in brackets represent the standard error of the means.

In winter, cortisol concentrations increased significantly during the restriction treatment for the juveniles (25–40% increase from 82.91 ± 6.51 ng mL^{-1} at B4, all $p < 0.01$), but changes were not significant for subadults (all $p > 0.05$, Table 3). Overall, juveniles had greater cortisol concentrations than subadults at the end of the restriction ($p = 0.04$). For the subadults, cortisol concentrations were only elevated above baseline concentrations in the middle of the controlled refeeding ($p = 0.05$, otherwise all $p > 0.08$), while free cortisol concentrations increased throughout the winter experiment (from 10.39 ± 4.67 ng mL^{-1} at B4 to 30.72 ± 12.12 ng mL^{-1} at R4, all $p < 0.01$). Juveniles showed a five-fold increase in concentrations of free cortisol during winter restriction compared with baseline concentrations (all $p < 0.001$; Fig. 1a, b). During the controlled refeeding, concentrations tended to decrease slightly but were still greater than the baseline values ($p > 0.01$). Free cortisol concentration were greater for the juveniles compared with the subadults only at the end of the restriction ($p = 0.05$).

3.2.3. Relationships to body mass, body composition and blood metabolites

Concentrations of cortisol and free cortisol were negatively related to changes in body mass of the sea lions in winter (both $p < 0.001$) while only free cortisol concentrations were related to changes in body mass in summer ($p = 0.01$). Diet group, age of the animals, and season did not affect the relationships between glucocorticoid concentrations and body mass (all $p > 0.09$). Cortisol and free cortisol were significantly related to body condition (body fat as a percent of total mass [TBF%], both $p < 0.03$) independent of season and diet group (all $p > 0.07$), but were not related to changes in body condition ($p > 0.1$, Fig. 2a, b). However, the slope of the relationship between cortisol and body condition was greater for juveniles than subadults ($p = 0.009$). Finally, NEFA was related to free cortisol and cortisol concentrations in winter (all $p < 0.03$) but not in summer (all $p > 0.39$).

3.3. Insulin-like growth factor-I

3.3.1. Effects of diet composition

Diet group never affected changes in IGF-I concentrations in either season (all $p > 0.10$).

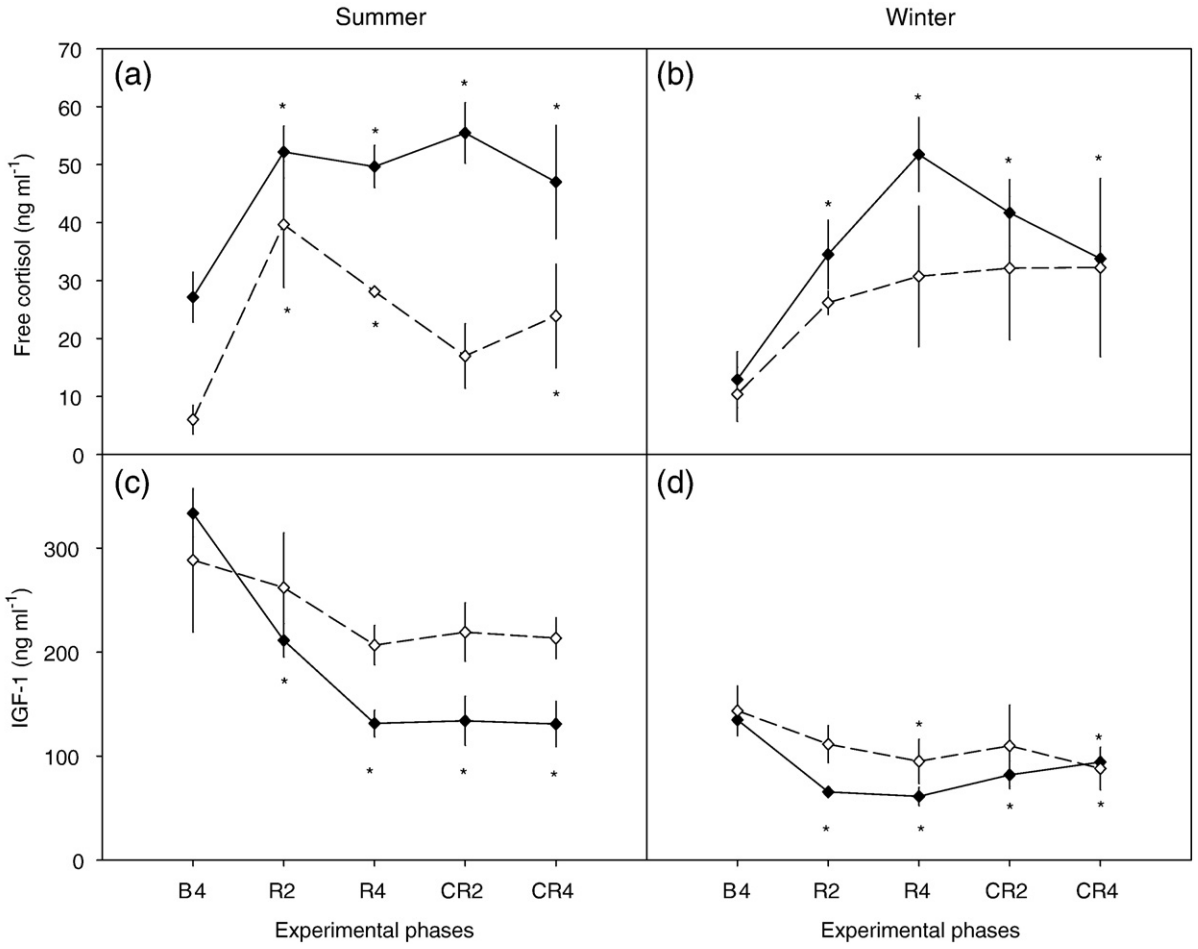

Fig. 1. Mean ± SE serum concentration of free cortisol and IGF-I in eight female Steller sea lions segregated by age class (filled diamonds: juveniles and open diamonds: subadults) measured at the end of the baseline (B4), and after 2 and 4 weeks of restricted food intake (R2 and R4) and a controlled refeeding (CR2 and CR4) period. The two graphs on the left (a and c) represent the data collected during the summer 2005 experiment and the two graphs on the right (b and d) during the winter 2006 experiment. The asterisks indicate significant within-group differences compared to the respective B4 measurement.

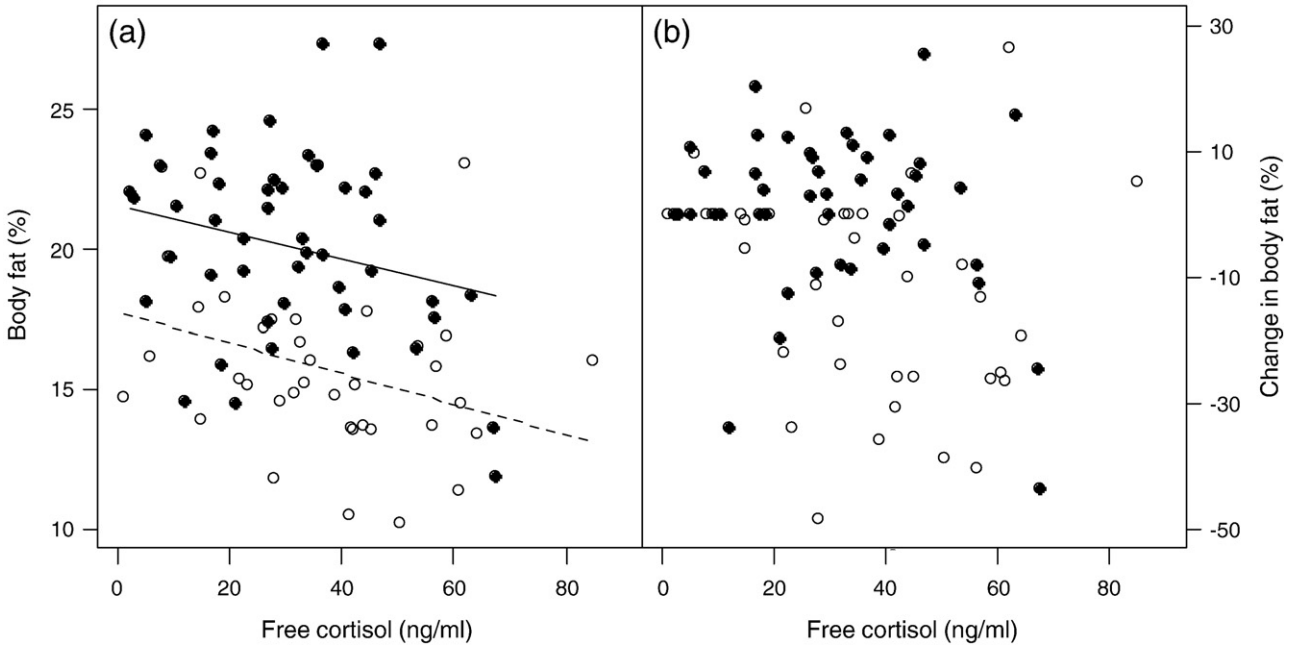

Fig. 2. Relationships between blood concentrations of free cortisol (ng mL^{-1}) and body condition (% of fat in the body) (a), and between free cortisol and relative changes in body fat (compared to baseline concentrations) (b) in the 8 female Steller sea lions during the summer (open circles and dashed line) and winter (closed circles and plain line) experiments. The linear relationships were fitted using mixed-effects models. There were no seasonal difference in the relationships' slopes in (a) ($p = 0.82$), but intercepts were statistically different ($p = 0.0005$).

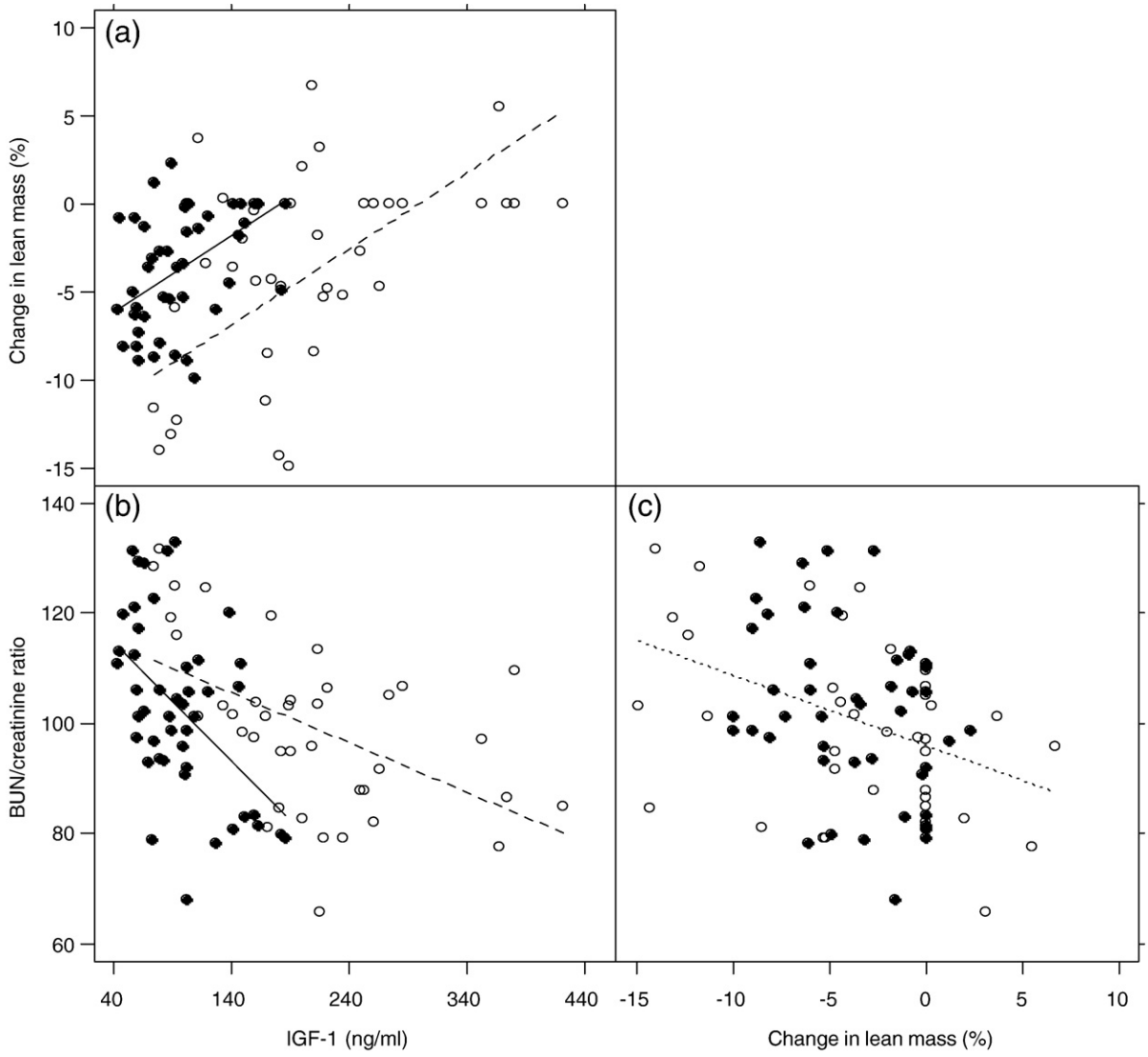

Fig. 3. Relationship between change in lean body mass and serum concentration of IGF-I (ng mL^{-1}) (a), the blood urea nitrogen [BUN]/creatinine ratio and serum concentration of IGF-I (ng mL^{-1}) (b) and BUN/creatinine ratio and changes in lean body mass (%) (c) in eight female Steller sea lions in summer 2005 (open circles and dashed lines) and winter 2006 (closed circles and plain lines). Slopes and intercept were similar between season for graph (c), represented by a unique dotted line. The linear relationships were fitted using linear mixed-effects models and were all significant (all $p<0.01$). Slopes in graphs (a) and (c) were similar between seasons (both $p>0.43$) but were different in graph (b) ($p=0.04$).

3.3.2. Effects of age and season

Absolute concentrations of IGF-I in each phase were overall greater in summer than in winter ($p=0.0001$), and the relative decline during the restriction was more pronounced in summer as well (Fig. 1c and d and Table 3, all $p<0.02$). In summer, changes in IGF-I during the restriction and the subsequent controlled refeeding were significantly more pronounced for the juveniles than for the subadults (all $p<0.0001$; Fig. 1c). Concentrations of IGF-I in juveniles were less than baseline values (333.39±22.56 ng mL^{-1} at B4) throughout the experiment (60% decrease at R4 and throughout the refeeding, all $p<0.0001$), while in the subadults the observed trend was also a decrease but not significant (all $p>0.07$). As a result, absolute concentrations of IGF-I were less for juveniles than subadults at the end of the restriction and during the controlled refeeding (all $p<0.05$).

In winter, concentrations of IGF-I declined during the restriction in both juveniles and subadults compared with baseline (all $p<0.002$) but at a faster rate in juveniles ($p=0.06$, Fig. 1c, d and Table 3). In terms of absolute value, juveniles showed lower concentrations of IGF-I than subadults only in the middle of the restriction (juveniles: 65.46±4.11 ng mL^{-1}, subadults: 111.42±17.75 ng mL^{-1}, $p=0.01$; other phases: $p>0.13$). During the controlled recovery, IGF-I concentrations were still lower than baseline values ($p=0.03$ at CR1 and $p=0.0005$ at CR2), but changes in concentrations were greater for juveniles compared to subaldults ($p=0.02$).

3.3.3. Relationships to body mass, body composition and blood metabolites

IGF-I was positively related to body mass in both summer and winter (both $p<0.0001$). These relationships were different between seasons and between age groups (all $p<0.001$). Changes in concentrations of IGF-I were also positively related to changes in lean body mass in both seasons (both $p<0.002$, Fig. 3a) but relationships were similar between season, age, and diet groups (all $p>0.8$) and the intercept was greater in winter ($p=0.001$). IGF-I concentrations were not related to body condition (% of body fat, all $p>0.08$).

Changes in BUN:creatinine ratio were negatively related to changes in IGF-I concentrations both in summer and winter (both $p<0.002$; Fig. 3b) and the winter slope was significantly less than summer one ($p=0.01$). Finally, IGF-I was negatively related to free cortisol concentration, and positively related to TT4 in both seasons (both $p<0.01$). The slopes of all these relationships were similar between seasons (both $p>0.18$), but not their intercepts (both $p<0.007$). IGF-1 was only related to TT3 in summer ($p<0.0001$) but not in winter ($p=0.58$).

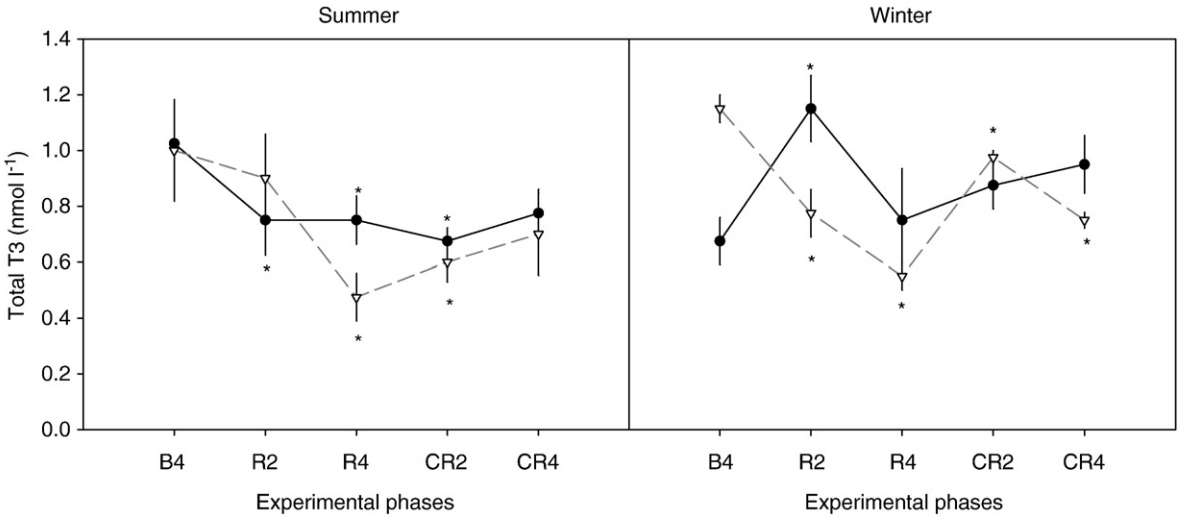

Fig. 4. Mean ± SE serum concentration of total T3 (nM) in eight female Steller sea lions segregated by diet groups (circles: group H, triangles: group P) in summer 2005 (a) and winter 2006 (b). Measurements were taken at the end of the baseline (4th week, B4) and at week 2 and 4 of the restriction (R) and of the controlled refeeding (CR). The asterisks indicate significant within-group differences compared to respective B4 measurement.

3.4. Thyroid hormones

3.4.1. Effect of age

Age never affected absolute concentrations or relative changes of thyroid hormones compared to the baseline measurement during the summer and winter experiments (all $p>0.35$, Fig. 5).

3.4.2. Effects of diet composition and season

In summer, relative changes in concentrations of TT3 during the restriction and the controlled refeeding were similar for both diet groups (all $p>0.20$; Fig. 4). Concentrations of TT3 were significantly less than baseline concentrations (1.01 ± 0.10 nM) from the end of the restriction (R4: −35% at 0.61 ± 0.08 nM) until the end of the experiment (0.74 ± 0.08 nM at CR4, all $p<0.02$). Concentrations of TT4 and fT4 were not affected by diet group (all $p>0.09$) or by energy restriction and the subsequent recovery in the summer (all $p>0.21$, baseline concentrations TT4: 14.00 ± 0.80 nM and fT4: 25.75 ± 2.52 pM). Overall, the TT3/TT4 ratio decreased significantly during the restriction in summer (from 0.073 ± 0.008 at B4 to 0.054 ± 0.008 at R4, $p<0.002$) and did not return to baseline until the end of the controlled refeeding (0.065 ± 0.009 at CR4, $p=0.27$).

In winter however, diet group significantly affected changes in TT3 concentrations during the restriction ($p=0.0005$, Fig. 4). Concentrations of TT3 for Group P decreased during the restriction relative to baseline values (−50% decrease from 1.15 ± 0.05 nM at B4 to 0.55 ± 0.05 nM at R4) and although still less than baseline concentrations, increased slightly during the controlled refeeding, (−35% decrease and 0.75 ± 0.03 nM at CR4, all $p<0.03$). Concentrations of TT3 for Group H were generally stable compared with the baseline (0.68 ± 0.09 nM at B4, all $p>0.08$), except for a temporary increase in the middle of the restriction (65% increase at 1.15 ± 0.12 nM, $p=0.01$), when TT3 concentrations were greater for Group H compared with for Group P ($p=0.0001$). Neither fT4 nor TT4 were affected by diet group in winter (all $p>0.09$). Concentrations of fT4 remained constant (21.87 ± 1.81 pM at B4 and 25.83 ± 3.88 pM at R4) but TT4 concentrations decreased (~25%) during the restriction (from 12.63 ± 1.18 nM at B4 to 9.38 ± 1.64 nM at R4, $p<0.04$) before returning to baseline concentrations during the controlled refeeding (12.25 ± 1.13 nM at R4, $p>0.05$). Finally, concentrations of TT3/TT4 increased in the middle of the restriction (from 0.075 ± 0.010 at B4 to 0.130 ± 0.032 at R2, $p=0.03$) but returned to baseline concentrations at the end of the restriction and remained stable during the controlled refeeding (0.073 ± 0.008 at CR2, all $p>0.30$). Group P had lower TT3/TT4 ratios at the end of the restriction in both seasons compared with Group H (both $p<0.01$). Overall, changes in thyroid hormones were similar between season at all measurement points (all $p>0.22$) except for TT3 and TT3/TT4 ratio at R2 (both $p<0.02$) (Fig. 5).

3.4.3. Relationship with metabolic rates

Daily metabolic rate (DMR) and standard metabolic rate in air (SRM_A) were measured in the course of the summer and winter experiments and results were published in Jeanniard du Dot et al. (In press). Neither TT3, TT4, nor fT4 showed a significant relationship with the measured DMR or SRM_A (all $p>0.1$).

3.5. Blood metabolites

3.5.1. Effects of age

Age of the animals did not alter changes in BUN:creatinine ratio, NEFA, or β-HBA during the restriction periods or the controlled refeedings (all $p>0.10$).

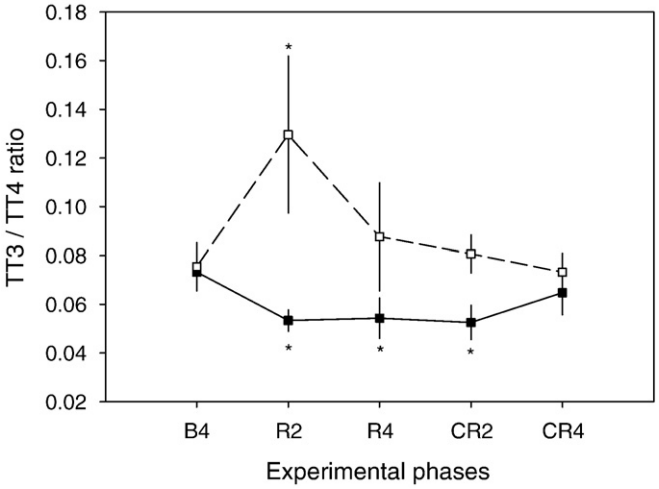

Fig. 5. Mean ± SE changes in ratio of total T3 (TT3)/total T4 (TT4) in eight female Steller sea lions in summer 2005 (filled squares) and winter 2006 (open squares). Measurements were taken at the end of the baseline (4th week, B4) and at week 2 and 4 of the restriction (R2 and R4) and of the controlled refeeding (CR2 and CR4). Asterisks indicate the within-group differences compared to the respective B4 measurements.

3.5.2. Effects of diet composition and season

In summer, blood parameters were not influenced by the fish type animals were eating (all $p>0.06$). BUN:creatinine ratios were greater than baseline concentrations only in the middle of the controlled refeeding (109.88±4.71 compared with baseline concentration: 96.57±3.43, $p=0.01$), but not during the energy restriction period (94.49±6.50 at R4). This was due to an increase in BUN (from 8.00±0.33 mM at B4 to 9.00±0.45 mM at CR2) rather than a decrease in creatinine (83.88±8.25 μM at B4 to 82.50±4.32 μM at CR2). In addition, restriction triggered a decrease in concentrations of NEFA in summer (all $p<0.02$) for all animals, but the decrease was greater ($p=0.04$) for Group H (1.02±0.29 mM, at B4, 0.43±0.07 mM at R4 and 0.35±0.05 at CR4) than group P (0.47±0.07 mM at B4, 0.78±0.27 mM at R4 and 0.69±0.27 at DCR4). Finally, β-HBA concentrations remained stable at baseline concentrations (0.08±0.04 mM) throughout the summer experiment (0.03±0.02 both at R4 and CR4, all $p>0.10$).

In winter however, diet composition had a significant impact during the restriction treatment on concentrations of BUN/creatinine (R2: $p=0.02$ and R4: $p=0.03$). Animals in Group P displayed an increase in BUN/creatinine ratio during the restriction (28% increase from 90.12±5.9 during the baseline to 112.9±6.6 after 2 weeks of restriction $p=0.05$ and 42% at 126.2±2.9 after 4 weeks $p=0.005$) and a following decrease during the controlled refeeding to return to baseline concentrations (95.4±6.0 at CR4). The increase in BUN/creatinine ratio for group P during the restriction was due to both a decrease in creatinine and an increase in BUN from 95.25±5.68 μM and 8.50±0.27 mM at B4 to 80.50±4.23 μM and 10.10±0.82 mM respectively. Ratio of BUN/creatinine for Group H stayed constant throughout the experiment (95.37±8.5 at B4, 105.4±3.9 at R4 and 107.1±7.7 at CR2; all $p>0.05$) due to concentrations of both BUN and creatinine remaining around baseline concentrations (8.10±0.38 mM and 87.00±9.64 μM, respectively). Absolute BUN/creatinine ratios were greater for Group P relative to Group H during the winter restriction (both $p<0.03$). Concentrations of NEFA increased during the winter restriction (50% increase from 0.42±0.06 mM at B4 to 0.65±0.10 mM at R4, $p=0.03$) and returned to baseline concentrations during the controlled refeeding (0.54±0.10 mM at CR4, $p=0.22$), with no significant difference between diet groups ($p=0.62$). Finally, 75% of the blood samples tested in winter for concentrations of β-HBA were too close to the detection limit of the measurement kits (detection limit: 0.01 mM) to evaluate concentrations accurately. Consequently statistical analysis was not possible on this parameter in winter.

3.5.3. Relationships to body mass and body composition

Changes in BUN/creatinine concentrations were negatively related to changes in lean body mass both in summer ($p=0.02$) and in winter ($p>0.005$) with no significant effect of diet group, age, or season (all $p>0.1$, Fig. 3c). Neither changes in NEFA nor β-HBA were related to changes in body fat ($p>0.07$).

4. Discussion

4.1. Endocrine changes during mild energy restriction

Concentrations of glucocorticoids, IGF-I, and thyroid hormones are known to vary with energy intake, quality and quantity of food, sex, and age as well as external variables such as season and temperature in mammals and birds (for clarity purposes, expected and observed trends in blood parameter concentrations during our experiments are summarized in Table 1) (Eales, 1988; Kitaysky et al., 2001a; Romero, 2002; Oki and Atkinson, 2004; Reeder and Kramer, 2005; Myers et al., 2006). Among their functions, these hormones are linked to regulation of body homeostasis, and lipid and protein metabolism to optimize fitness of animals during suboptimal conditions (Ward and Armitage, 1981). In the context of the decline of Steller sea lions, it is essential to understand the relationship between variation in energy budgets and endocrine factors to help interpret physiological effects of potential chronic nutritional stress. Hormones involved in regulation of nutritional physiology could also be used as health bioindicators of sea lions in the wild.

In the restriction phase of our experiment, energy intake of all the sea lions was reduced to a level that induced significant body mass loss (Table 1). Changes in glucocorticoid concentrations represent a major endocrine response to stressors, including nutritional stress, in mammals and birds (Bergendahl et al., 1996; Ortiz et al., 2001b) and somatotropic hormones such as IGF-I are the main inducers of growth in terrestrial mammals (Hornick et al., 2000; Lupu et al., 2001). The two groups of hormones were expected to be affected by nutritional stress in sea lions, reflecting control mechanisms for body homeostasis during periods of mass loss. The increase in cortisol and free cortisol concentrations and the decrease of IGF-I observed in the sea lions were similar to those observed for other mammals during nutritional stress (Mosier, 1986; Buonomo and Baile, 1991; Ortiz et al., 2001a).This supports the notion that IGF-I and free cortisol are involved (most likely cooperatively) in the control of body mass homeostasis of sea lions in both summer and winter (Goodman and Knobil, 1961; Ashwell-Erickson et al., 1986) and could thus be used as indicators of general stress in Steller sea lions.

Although the sea lions lost mass at the same rate during the energy restrictions, the two diet groups lost body parts (lipid mass versus lean mass) in different proportions during mass loss (as calculated from differential changes in body composition). There was, however, no difference in changes in concentrations of IGF-I (preservation agents of body protein; Breier, 1999) and glucocorticoids (mobilizing energy stores through lipolysis; Ortiz et al., 2001a) between diet groups. This result indicates that hormone concentrations were not directly influenced by the type of fish eaten. However IGF-I was positively related to changes in lean mass both in winter and summer and to BUN/creatinine ratios. Similarly, free cortisol concentrations were related to absolute concentrations of body fat, as previously reported for other pinnipeds (Ortiz et al., 2001a, 2003; Guinet et al., 2004; Kumagai, 2004b), and to concentrations of NEFA, a by-product of lipid catabolism. This indicates that these two hormones could be used as biomarkers of changes in lean and lipid mass respectively. In addition, even if hormonal changes were not directly linked to type of diet fed to the sea lions, they were linked to changes in body composition that were themselves attributable to the experimental diet. Pairing IGF-I, found to be a valid indicator of changes in lean mass, with free cortisol an indicator of lipid catabolism, could thus provide insights on the strategy of fuel utilization (lipid or protein) during nutritional stress that either of these hormones could not achieve alone. Among the blood metabolites measured, only the BUN/creatinine ratio was found to reflect differences in body composition changes between the two diet groups and might be used in extreme cases as a bioindicator given that blood concentrations decreased in winter for Group P when the animals lost up to 80% of their body mass as lean mass (potentially from muscle). Assessing the proportion of lean mass versus lipid mass catabolised during energy restriction is useful to assess the long term consequences of high- versus low-energy density diets in sea lions, especially if animals predominantly lose lean mass which can impair organ integrity and ultimately survival and reproduction of the animals on a chronic basis.

It is also important to consider age of the animals and season while interpreting IGF-I and glucocorticoid concentrations, as both parameters were found to impact hormones concentrations. Relative decreases in IGF-I during periods of energy restriction were greater in summer for juvenile sea lions, even though changes in body mass and body composition during the restriction were not age-dependant. Furthermore, absolute concentrations of free cortisol were greater for juveniles in summer, while relative changes in free cortisol during restriction, compared to respective baseline concentrations, were more intense for juveniles than subadults during the winter energy restriction. This may suggest that greater endocrine responses, at least for free cortisol and IGF-I, are necessary for juveniles to preserve body homeostasis and body

composition to the same level as subadults. Younger animals may be less sensitive to changes in hormone concentration compared with adults (Govoni et al., 2003) which would require greater changes in hormones concentrations to elicit a response to stress. Although an increase in glucocorticoids is part of the natural acute defence response, long term elevated concentrations of stress hormones can be detrimental, particularly for development and cognitive capacities of young animals (Kitaysky et al., 2006). If wild Steller sea lions experience mild restriction at an early age (as might happen when eating low-energy density fish on a chronic basis) that results in increased concentrations of stress hormones, then growth, diving and foraging capacity and the immune system could be impaired (St Aubin and Dierauf, 2001; Kitaysky et al., 2003; Reeder and Kramer, 2005; Richmond et al., 2006), particularly as young animals are more likely to suffer from nutritional stress during suboptimal conditions since they require a higher mass-specific energy intake than older ones (Kleiber, 1975; Winship et al., 2002).

Thyroid hormones are a third set of hormones known to be involved in regulation of body homeostasis during periods of nutritional stress. Blood concentrations of thyroid hormones play an important role in basal metabolic expenses and metabolic thermogenesis (Renouf and Noseworthy, 1991; Silva, 1995; Haulena et al., 1998) and were expected to decrease during the energy restrictions as a mechanism to save energy (Azizi et al., 1979; Eales, 1988; Kelly, 2000; Diez et al., 2004). Concentrations of TT3 decreased slightly during the energy restriction in summer and TT4 in winter. However, fT4 concentrations did not significantly change during the experiments. Unlike data in other pinnipeds and woodchucks (Young, 1984; Ashwell-Erickson et al., 1986; Boily, 1996), none of the thyroid hormones were correlated to either measure of metabolism (DMR and SMR_A). This suggests that, even if changes in thyroid hormones were linked to changes in basal metabolic rates (not measured), responses may not have been severe enough to impact standard and daily metabolic rates. However, the down regulation of deiodination of T4 into T3 (as observed from TT3/TT4 data) and the decrease of TT3 concentrations in summer likely reflected a decrease in the anabolic activity of the thyroid hormones even though we were not able to quantify it. Changes in thyroid hormones were overall less pronounced than anticipated. However, we did not measure free T3, the most metabolically active hormone nor reverse T3 which plays an important role in the regulation of thyroid hormone activity during nutritional stress (O'Brian et al., 1980; Kelly, 2000). Changes in their concentrations could show a more pronounced role of thyroid hormones in Steller sea lions energy regulation than our results indicate.

4.2. Endocrine changes during the controlled refeeding

The endocrine changes that occur during the energy restriction phase establish the hormonal state at the beginning of the refeeding phase. These altered concentrations likely modify the capacity of organisms to recover from nutritional stress (Mosier, 1986; Boersma and Wit, 1997; Hornick et al., 2000). In our study, the sea lions displayed a compensatory increase in body mass post-restriction in winter but not in summer (for more details, see Jeanniard du Dot et al., 2008). The hormonal control of compensatory growth is well documented in domestic animals and humans (Mosier, 1986; Boersma and Wit, 1997; Hornick et al., 2000) and involves cooperative changes in IGF-I, glucocorticoid, and thyroid hormones (Boersma and Wit, 1997). Compensatory growth usually results from an important increase in production of IGF-I upon refeeding, especially if growth hormone [GH] concentrations were elevated during the restriction. Simultaneous increase in concentrations of GH and IGF-I, works synergistically to increase anabolism providing energy for structural growth and/or energy storage (Mosier, 1986; Van Den Brande, 1986; Boersma and Wit, 1997).

In our study, winter concentrations of IGF-I increased to baseline values upon refeeding when compensatory growth was observed in combination with an increase in thyroid hormones and decrease in cortisol. The collaboration between the increase in energy intake and the signals from different endocrine systems to increase tissue synthesis might have facilitated the occurrence of the observed spurt of growth in the sea lions in winter, in the same way described for terrestrial mammals (Hornick et al., 2000). Whatever the mechanisms, there appears to be a priority for Steller sea lions to regain their 'optimal' mass in winter compared to summer, potentially for reproductive or thermoregulatory reasons (Kleiber, 1975; Pitcher et al., 1998).

In summer however, this phenomenon did not occur. It is unclear why hormone concentrations did not reach pre-energy restriction concentrations in summer. Hormone concentrations are known to vary seasonally (Thissen et al., 1994; Romero, 2002; Myers et al., 2006) and could be linked to a greater structural growth in summer. If this was the case, then seasonal differences in compensatory growth could result from a priority to resume structural growth (to the detriment of body energy content which might not lead to a mass increase) in summer and a priority for energy accumulation (which increases mass) in winter (Jeanniard du Dot et al., 2008). In addition, hormone concentrations remained similar to those attained at the end of the energy restriction period in summer, even though the animals stopped losing mass. This means that under different levels of energy intake (restriction and refeeding) the same concentrations of hormones were linked to different levels of catabolic response and body mass change (mass loss during restriction, maintenance during refeeding in summer). While the increase in nutrient intake in the controlled refeeding period did not elicit a hormonal response sufficient to return to pre-experimental body mass, changes in nutrient intake could be linked to changes at other levels within the endocrine system, such as a difference in tissue sensitivity to the hormones that could have facilitated the stop of mass loss (Wingfield and Kitaysky, 2002). For example, circulating IGF-I is bound to IGF binding proteins (IGFBP), which can alter IGF-I availability and therefore biological activity in both positive and negative ways (Jones and Clemmons, 1995). Therefore, even though the overall IGF-I concentrations did not increase as expected with refeeding in summer, changes in IGFBP could account for the differential response of sea lion (Govoni et al., 2003).

It is also interesting to note that during the one-month controlled refeeding periods in both seasons, concentrations of glucocorticoids and IGF-I never reached the pre-restriction concentrations, even if in winter the trends especially for juveniles were indicative of the system returning to initial baseline concentrations. A comparison of results between a previous study on the same animals that were fed ad-libitum food of high energy fish after severe energy restriction (Kumagai, 2004a; Kitaysky et al., 2007) showed that the return to baseline concentrations of glucocorticoid was rapid and complete. In our study however, the amount of food the animals were given during the controlled refeeding was equal to the energy content and composition of food they were given before the energy restriction. In this case, the animals did not recover at all during the summer and the hormone concentrations did not complete their return to baseline during the one month refeeding in winter. This indicates that there might be a cumulative effect of energy restriction/refeeding on the nutritional axis of sea lions that is highly linked to the quantity and type of food available to sea lions, in addition to a seasonal effect. Importantly, in the context of the nutritional stress hypothesis, if sea lions do not have access to ad-libitum high energy fish after a period of energy restriction such as in this study, the recovery capacity might be impaired or greatly delayed, especially in summer time.

5. Conclusions

All the hormones discussed above are intricately related to one another to create a complex web that regulates nutritional and energetic status (Robson et al., 2002). Absolute hormone concentrations provide

only a reductive snapshot of the very complex and dynamic endocrine regulation of energy budget (Eales, 1988). Their actions are also subject to changes in sensitivity at the organ level, either on a seasonal basis as part of the natural life cycle (Romero, 2002), or as a security control in case of chronic elevated hormone concentrations (St Aubin and Dierauf, 2001). Consequently, it is extremely difficult to assess the dominant regulatory systems and the endocrine changes related to the physiological responses observed. The results of our study show that a panel of hormones from different hormone axes such as concentrations of glucocorticoids (mostly free cortisol) and IGF-I can nevertheless be useful tools to understand and detect the general occurrence of a mild nutritional stress in sea lions, and to some extent the energetic strategy (lipid versus protein mass catabolism) the animals chose to cope with this nutritional stress and the subsequent refeeding. These data also provide clues on how age and season affect the response of the animals to energy restriction. Determining bioenergetic strategies of sea lions when faced with different nutritional condition might help in understanding how well the animals cope with changes in their environment when faced with unpredictable shortage of energy intake.

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