

GH and ghrelin increase with fasting in a naturally adapted species, the northern elephant seal (*Mirounga angustirostris*)

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Abstract

After nursing, pups of the northern elephant seal (*Mirounga angustirostris*) are approximately 46% body fat and rely almost entirely on the oxidation of their large fat stores to sustain their metabolism for the ensuing 8–12 week postweaning fast, which is a natural component of their life history. Thus, fasting pups provide an ideal opportunity to examine the hormonal alterations associated with prolonged food deprivation in a naturally adapted model. Cortisol, ghrelin, glucagon, growth hormone (GH), insulin-like growth factor-I (IGF-I), insulin, blood urea nitrogen (BUN), glucose and non-esterified fatty acids (NEFA) were examined in 20 male and 20 female pups blood sampled early (< 1 week postweaning) and late (6–8 weeks postweaning) during the fast. Mean cortisol, ghrelin, GH, and glucagon increased 1.8-, 1.8-, 1.4-, and 2.3-fold between early and late periods, while mean IGF-I

and insulin decreased 97% and 38%, respectively. NEFA increased 2.3-fold, while BUN and glucose decreased 46% and 11%, respectively. NEFA was significantly and positively correlated with cortisol and GH; individually; however, when the relationship was examined as a multiple regression the correlation improved suggesting that cortisol and GH act synergistically to promote lipolysis during the fast. GH and BUN were negatively and significantly correlated between early and late fasting suggesting that GH may promote protein sparing as well. The decrease in glucose may be responsible for stimulating glucagon, resulting in the maintenance of relative hyperglycemia. The increases in cortisol, ghrelin, glucagon, and GH suggest that these hormones may be integral in mediating the metabolism of seal pups during prolonged fasting.

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Introduction

Fasting for prolonged (2–3 months) periods is a natural component of the life history of the northern elephant seal (*Mirounga angustirostris*) regardless of age. After birth, pups nurse for approximately one month during which time they increase their body fat stores to approximately 45–50% of body mass (Ortiz *et al.* 1978, Rea & Costa 1992, Houser & Costa 2001, Ortiz *et al.* 2001a). Upon weaning, pups abstain from food and water for 2–3 months utilizing their extensive fat stores as the primary source of energy during the postweaning fast (Rea & Costa 1992, Houser & Costa 2001). Interestingly, pups also maintain a condition of relative hyperglycemia (> 7 mM) during the fast (Costa & Ortiz 1982, Keith & Ortiz 1989, Ortiz *et al.* 2001b) even though glucose metabolism contributes only about 1% to the pups' metabolic rate (Keith & Ortiz 1989). However, hormonal regulation of fat metabolism and hyperglycemia is not well described in seals or marine mammals in general.

Despite the large body fat stores, leptin, the adipocyte-derived hormone commonly implicated in energy balance

and fat metabolism in mammals (Considine & Caro 1997, Ahima & Flier 2000) is not correlated with body fat mass and does not appear to play an integral role in regulating energy balance in elephant seal pups (Ortiz *et al.* 2001a,b, 2003). Paradoxically, thyroid hormones increased during the fast with these changes more closely reflecting an alteration in hormone clearance and metabolism than active secretion, and thus do not likely contribute to the regulation of metabolism in fasting pups (Ortiz *et al.* 2001b, 2003). The linear increase in circulating cortisol observed during the fast suggests that this hormone, more so than any others previously examined, contributes to the regulation of fasting metabolism in postweaned pups (Ortiz *et al.* 2001b). However, the effects of prolonged fasting on other hormones such as ghrelin, growth hormone (GH), and insulin-like growth factor-I (IGF-I) that are commonly associated with regulating energy balance and metabolism, have yet to be examined in marine mammals, which are adapted to protracted periods of food deprivation.

The deprivation of food is generally associated with increases in circulating ghrelin (Toshinai *et al.* 2001),

GH (Vance *et al.* 1992, Straus 1994), glucagon (Boyle *et al.* 1989) and decreases in IGF-I (Straus 1994, Thissen *et al.* 1994) and insulin (Boyle *et al.* 1989). Ghrelin is a gastric-derived protein that is known to stimulate the secretion of growth hormone (Kojima *et al.* 1999, Seoane *et al.* 2000, Takaya *et al.* 2000), stimulate feeding (Wren *et al.* 2000, Nakazato *et al.* 2001), and play a role in carbohydrate metabolism (Broglia *et al.* 2001, Toshinai *et al.* 2001). Aside from the growth promoting effects of GH mediated through IGF-I (for review see Kopchick & Andry 2000), GH also stimulates lipolysis (Goodman 1968, Fielder & Talamantes 1987) and conserves protein (Nørrelund *et al.* 2001). In mammals, food deprivation is usually associated with an elevation in glucocorticoids, which induce lipolysis and provide substrates for subsequent metabolism (Bergendahl *et al.* 1996). Reduced insulin and increased glucagon secretion provide the primary counter-regulatory mechanisms responsible for alleviating fasting-induced hypoglycemia (Boyle *et al.* 1989).

The effects of food deprivation or energy restriction on cortisol, ghrelin, GH, glucagon, insulin, and IGF-I have been well established in humans and rodents; however, unlike humans and rodents, most marine mammals are adapted to prolonged periods of food deprivation and regularly experience such fasting bouts as a natural component of their life history. Thus, this group of mammals provides a unique model from which to examine the effects of food deprivation in an adapted species. However, very little information exists in this regard. Therefore, the present study was conducted to describe the hormonal and biochemical responses to prolonged fasting as well as to examine the potential contribution of these hormones on the increase in fat oxidation, decrease in protein catabolism, and maintenance of fasting hyperglycemia in northern elephant seal pups.

Materials and Methods

All methods were reviewed and approved by the University of California Santa Cruz Chancellor's Animal Research Committee. The present study was conducted in conjunction with other studies on the metabolism and endocrinology of northern elephant seal (NES) pups (Ortiz *et al.* 2001a).

Animals

Forty NES pups (20 males, 20 females) from Año Nuevo State Reserve (approximately 30 km north of Santa Cruz, CA, USA) were studied. Mother-pup pairs were individually identified in order to determine the date of weaning. A pup was considered weaned when its mother was not seen on the subsequent day. Body mass and blood samples were obtained early (<1 week postweaning) and late (6–8 weeks postweaning) during the pups' natural,

postweaning fast. Animals were left in their natural habitat between sampling periods.

Procedures and analyses

Procedures were similar at both the early and late sampling periods. Body mass was measured using a hanging-load cell suspended from a tripod. After weighing, pups were sedated with 0.01 cc tiletamine HCl and zolazepam HCl (Telazol; Fort Dodge Animal Health, Fort Dodge, IA, USA) kg/body mass so that a 16 gauge, 3.5-inch spinal needle could be inserted into the extradural spinal vein from which a blood sample was obtained and tritium infused for the estimation of body fat as previously detailed (Ortiz *et al.* 2001a). Blood (11 ml) was collected into an untreated vacutainer tube. After collection, blood tubes were placed on ice in a portable ice chest until they could be returned to the lab to be centrifuged, which was within 6 h. Blood samples were then centrifuged for 15 min (1500 g at 4 °C), and serum collected and frozen at –70 °C for later analyses.

Hormone concentrations were measured by either radioimmunoassay or enzyme immunoassay (IGF-I) using commercially available kits: ghrelin (rabbit anti-human; Phoenix Pharmaceuticals, Belmont, CA, USA), growth hormone (guinea pig anti-porcine), glucagon, insulin (guinea pig anti-porcine) (Linco, St Charles, MO, USA), and IGF-I (DSL, Webster, TX, USA). Samples were diluted 1:3 in assay buffer (pH=7.4) prior to determination of ghrelin. An acid-ethanol extraction of a 100 µl aliquot of sample was performed prior to the measurement of IGF-I using the manufacturer's protocol (DSL). Immunoreactivity of NES hormones in serum (or plasma for GH) was validated for each commercial antibody by examining the degree of parallelism between a serially diluted pool of NES serum (or plasma) and the standard curves (i.e. Fig. 1). For the GH assay, an additional pool of plasma from California sea lions (*Zalophus californianus*) was included in the validation for comparative purposes. Cortisol concentrations were reported previously in conjunction with a separate study (Ortiz *et al.* 2001a). Percentage recovery of exogenous hormone from pooled samples was consistently greater than 92%. All samples were analyzed in duplicate and run in a single assay with intra-assay percentage coefficients of variability of less than 11% for all assays. Blood urea nitrogen (BUN) and glucose were measured on a clinical autoanalyzer (Roche Diagnostics, Somerville, NJ, USA). Non-esterified fatty acids (NEFA) were determined by an enzymatic colorimetric method (Wako, Richmond, VA, USA).

Statistics

Initially, all measured parameters were compared by analysis of variance to examine the effect of gender at both

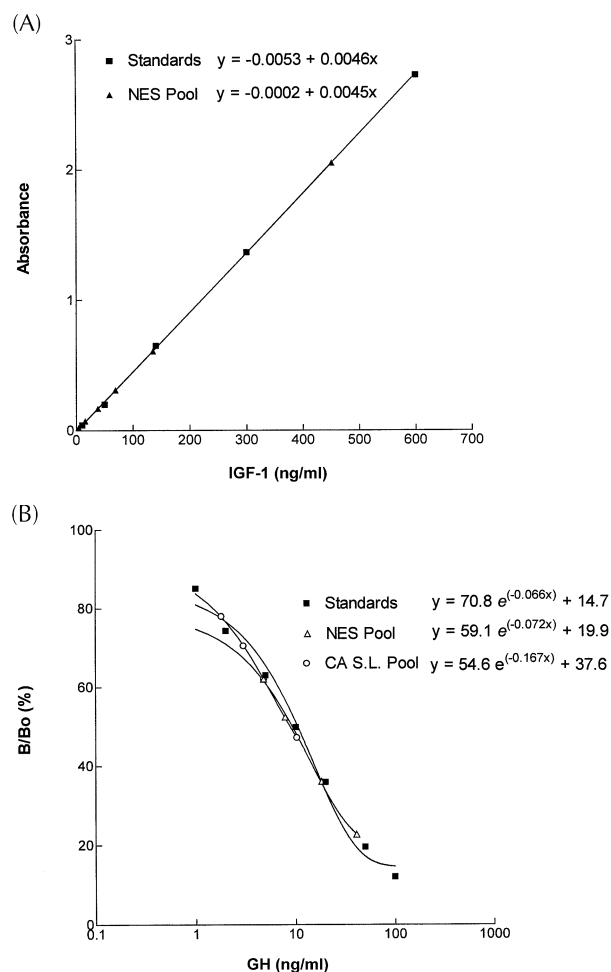


Figure 1 Representative estimation of the percentage of cross-reactivity between hormones in northern elephant seal serum and the commercial antibodies. (A) Serial dilution of pooled serum from northern elephant seals (NES) extracted for measurement of IGF-I using an enzyme immunoassay. (B) Serial dilution of pooled plasma from northern elephant seals (NES) and California sea lions (CA S.L.) using a radioimmunoassay.

early and late periods. If means (\pm S.E.) were not different between males and females at each sampling period, then values for each period were combined and a temporal effect was examined by paired *t*-test. Correlations were determined by simple regression. Differences were considered significant at $P < 0.05$. Statistical analyses were made using Statview (SAS Institute Inc., Cary, NC, USA).

Results

A gender effect was not detected at either sampling period for any of the parameters measured with the exception of insulin. Mean body mass was reduced 27.8% between

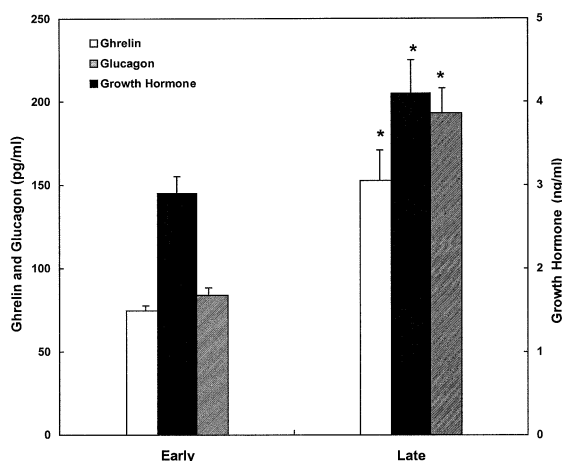


Figure 2 Serum ghrelin, glucagon, and growth hormone concentrations from 40 (20 male, 20 female) northern elephant seal pups sampled early (<1 week) and late (6–8 weeks) during their natural postweaning fast. * $P < 0.05$, significantly different from Early.

early (123.6 ± 2.5 kg) and late (89.2 ± 2.0 kg) periods associated with a 25.1% decrease in mean fat mass (early: 54.5 ± 1.5 kg; late: 40.8 ± 1.2 kg) during the same periods (Ortiz *et al.* 2001a). Cortisol ($P < 0.0001$) (Ortiz *et al.* 2001a), ghrelin ($P = 0.0016$), GH ($P = 0.0033$), and glucagon ($P < 0.0001$) were significantly increased 1.8-, 1.8-, 1.4-, and 2.3-fold respectively, between early and late periods (Fig. 2), whereas mean IGF-I (early: 93.2 ± 6.3 ng/ml; late: 3.1 ± 0.4 ng/ml; $P < 0.0001$) and insulin ($P < 0.0005$) were decreased 97% and 38%, respectively. Insulin concentrations were greater ($P < 0.03$) in females than males at both sampling periods (Fig. 3). BUN ($P < 0.0001$) and glucose ($P = 0.0045$) were significantly reduced by 46% and 11% respectively, while NEFA was significantly ($P < 0.0001$) increased 2.3-fold between early and late periods (Fig. 4). NEFA was significantly and negatively correlated with fat mass (Fig. 5) and positively correlated with both cortisol and GH concentrations (Fig. 6). The relationship between NEFA and the hormones improved when the correlation was examined as a multiple regression (NEFA = $0.47 + 0.06$ cortisol + 0.07 GH; $R = 0.480$; $P < 0.0001$). Also, GH and BUN (BUN = $4.56 - 0.21$ GH; $R = 0.267$; $P = 0.0165$) and GH and IGF-I (IGF-I = $71.55 - 6.62$ GH; $R = 0.241$; $P = 0.0315$) exhibited significant and negative correlations between early and late periods. Ghrelin and glucose were also significantly and negatively correlated (glucose = $8.18 - 0.004$ ghrelin; $R = 0.252$; $P = 0.0244$).

Discussion

Fasting for prolonged periods is a natural component of the life history of many mammals, and while the associated

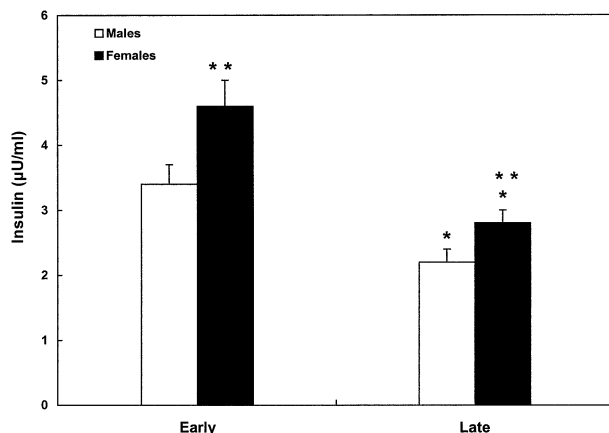


Figure 3 Serum insulin concentrations from 40 (20 male, 20 female) northern elephant seal pups sampled early (<1 week) and late (6–8 weeks) during their natural postweaning fast. * $P < 0.05$, significantly different from Early; ** $P < 0.05$, significantly different from males.

hormonal alterations in species not adapted to extended periods of food deprivation are well defined, such is not the case for naturally adapted mammals. Thus, fasting elephant seals provide a unique model for examining the hormonal and biochemical changes associated with prolonged food deprivation and the potential regulatory mechanisms of substrate metabolism. The present study identified a number of endocrine responses to protracted fasting and described their potential involvement in regulating metabolism during this period.

Northern elephant seal pups possess large body fat stores at weaning with approximately 95% of their fasting metabolism supported by the oxidation of fat (Castellini

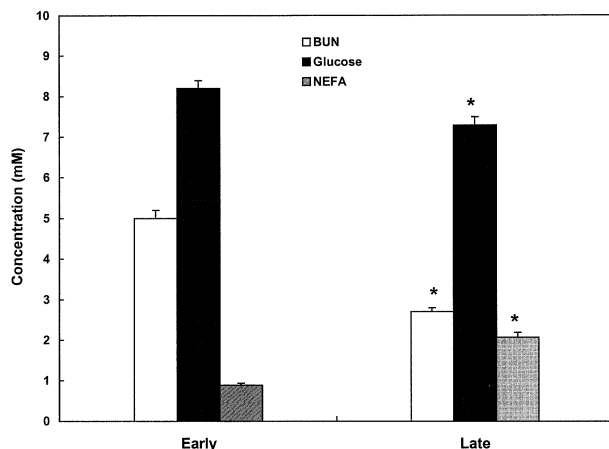


Figure 4 Blood urea nitrogen (BUN), glucose and non-esterified fatty acid (NEFA) concentrations from 40 (20 male, 20 female) northern elephant seal pups sampled early (<1 week) and late (6–8 weeks) during their natural postweaning fast. * $P < 0.05$, significantly different from Early.

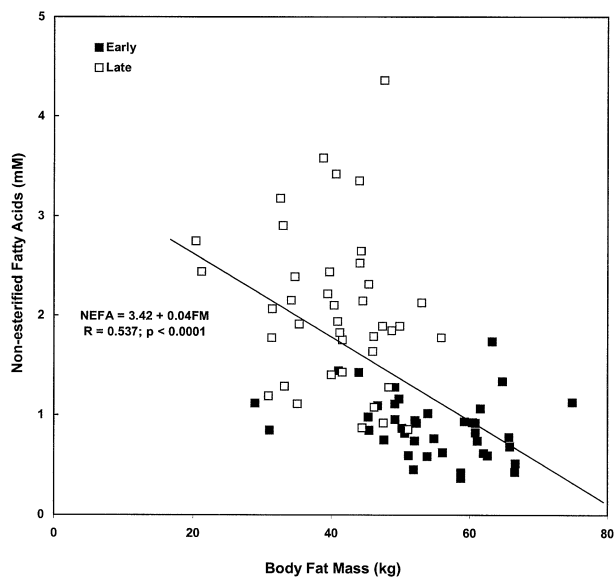


Figure 5 Correlation between non-esterified fatty acids (NEFA) and fat mass (FM) from 40 (20 male, 20 female) northern elephant seal pups sampled early (<1 week; ■) and late (6–8 weeks; □) during their natural postweaning fast. Regression was considered significant at $P < 0.05$.

et al. 1987, Rea & Costa 1992). Because the oxidation of body fat is the primary resource contributing to the animal's metabolism, body fat stores would be expected to be tightly regulated. However, very little data exists on the regulatory mechanisms of fat oxidation in this species during their naturally occurring fasts. We have previously suggested that the observed increase in circulating cortisol likely contributes to the increase in the mobilization of body fat, maintaining the animal's metabolism (Ortiz *et al.* 2001a,b). The inverse relationship between body fat mass and NEFA concentrations observed between early and late fasting periods suggests that the reduction in body fat is attributed to an increase in lipolysis resulting in elevated NEFA (Fig. 5). The positive relationships between NEFA and circulating cortisol and GH suggest that each of these hormones may be responsible for stimulating the increase in lipolysis during the fast. The hormonal relationship with NEFA improves when the correlation is examined as a multiple regression suggesting that cortisol and GH are likely acting synergistically to mediate this fasting-associated lipolysis. The interactions among glucocorticoids, GH and NEFA (or glycerol) are consistent with that previously observed *in vitro* using rat adipose tissue (Fain *et al.* 1965, Goodman 1968). Also, GH alone was reported to increase glycerol concentration *in vitro* using mice adipose tissue suggesting that GH induced an increase in lipolysis (Fielder & Talamantes 1987). Thus, cortisol and GH may act synergistically in fasting elephant seals to stimulate lipolysis and increase fatty acid oxidation.

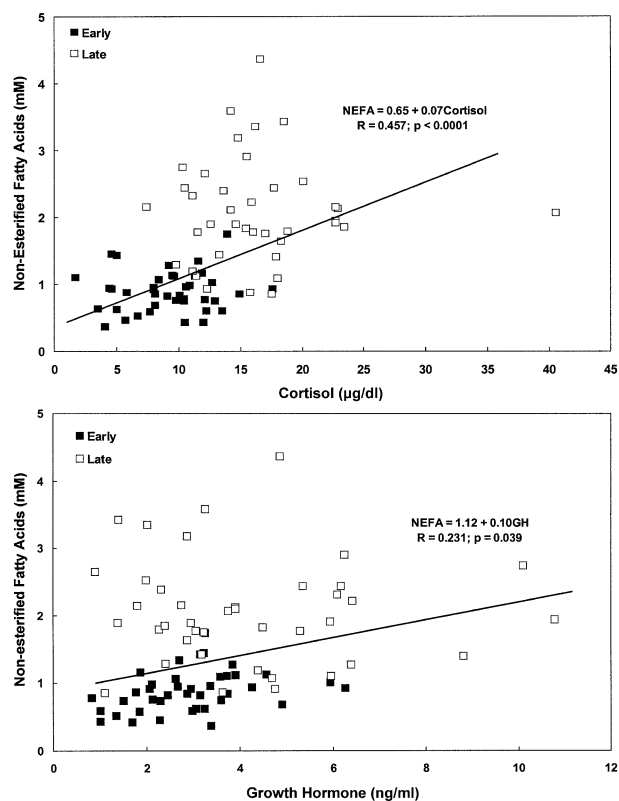


Figure 6 Correlations between non-esterified fatty acids (NEFA) and serum cortisol (upper panel) and growth hormone (GH) (lower panel) from 40 (20 male, 20 female) northern elephant seal pups sampled early (<1 week; ■) and late (6–8 weeks; □) during their natural postweaning fast. Regressions were considered significant at $P < 0.05$.

The increase in the oxidation of fat is associated with a concomitant decrease in protein catabolism in elephant seals (Pernia *et al.* 1980, Rea & Costa 1992, Adams & Costa 1993, Houser & Costa 2001). The decrease in BUN between early and late periods suggests that protein catabolism was reduced and is consistent with previous studies on elephant seals (Costa & Ortiz 1982, Adams & Costa 1993, Houser & Costa 2001). During short-term (40 h) food deprivation in GH-deficient humans, GH replacement stimulated protein synthesis and reduced urea excretion (Nørrelund *et al.* 2001). Treatment with recombinant human GH (rhGH) in GH-deficient patients also resulted in an increase in lean body mass and a concomitant decrease in fat mass (Salomon *et al.* 1989) suggesting that GH can regulate body composition by stimulating or maintaining lean tissue and increasing fat metabolism. The negative correlation between GH and BUN along with the positive correlation between GH and NEFA suggests that GH plays an integral role in regulating body composition during the postweaning fast by alleviating protein catabolism and increasing lipolysis.

Despite the increase in GH, IGF-I was drastically reduced between early and late fasting periods. Although GH is known to increase circulating IGF-I concentrations (Kopchick & Andry 2000), during conditions of energy restriction or protein deprivation IGF-I mRNA is down-regulated resulting in a decrease in circulating IGF-I (for reviews see Straus 1994, Thissen *et al.* 1994). This dissociation between GH and IGF-I during the fast may be attributed to a decrease in hepatic GH receptor expression (Straus & Takemoto 1990) resulting in GH resistance as previously suggested (Straus 1994, Thissen *et al.* 1994). Also, the negative correlation between GH and IGF-I suggests that the decrease in IGF-I may have alleviated the inhibition on GH releasing hormone (GHRH) and GH release resulting in an increase in circulating GH concentrations late in the fast.

The gastric-derived peptide, ghrelin, is an endogenous ligand for the growth hormone secretagogue receptor that stimulates the release of GH from the pituitary (Kojima *et al.* 1999, Seoane *et al.* 2000, Takaya *et al.* 2000). Although ghrelin and GH increased with fasting in the present study, whether the increase in GH was coupled to the increase in ghrelin could not be ascertained. Nonetheless, the fasting-associated increase in ghrelin in the present study is consistent with that observed in food-deprived rats (Toshinai *et al.* 2001). Conversely, ghrelin administration in rats stimulates an increase in food intake induced by upregulating mRNA expression of the orexigenic peptides, neuropeptide Y and agouti-related peptide (Kamegai *et al.* 2001, Nakazato *et al.* 2001). If a similar orexigenic pathway exists in elephant seal pups, then the increase in ghrelin may be a primary signal to cease fasting and to depart to sea to initiate feeding.

Ghrelin may also play an integral role in carbohydrate metabolism in mammals. Ghrelin induces hyperglycemia by reducing insulin secretion in humans (Broglia *et al.* 2001) and is upregulated by insulin-induced hypoglycemia in rats (Toshinai *et al.* 2001). The negative correlation between ghrelin and glucose in the present study is consistent with the insulin-induced hypoglycemia-mediated upregulation of ghrelin expression observed in rats (Toshinai *et al.* 2001), suggesting that ghrelin may play an important role in carbohydrate metabolism in fasting pups as well. Where reduced insulin secretion and hypoglycemia are normal consequences of food deprivation, increased glucagon release is an important counterregulatory mechanism to alleviate the decrease in glucose (Boyle *et al.* 1989). Changes in the insulin-glucagon counterregulatory mechanism in response to fasting-induced decrease in glucose in the present study are consistent with that observed in humans with the exception that elephant seal pups maintain a condition of relative hyperglycemia throughout the fast (Costa & Ortiz 1982, Keith & Ortiz 1989, Ortiz *et al.* 2001b) despite the decrease in glucose late in the fast, suggesting that elevated

blood glucose in these marine mammals may be an important adaptation required for other physiological processes such as diving and does not necessarily reflect a biomedical detriment.

Ghrelin concentrations in the present study are two- to eightfold lower than those reported for humans (Tschöp *et al.* 2001b) and one to three orders of magnitude lower than those reported for mice and rats (Toshinai *et al.* 2001, Tschöp *et al.* 2001a). Tschöp *et al.* (2001b) originally hypothesized that obese individuals would exhibit elevated concentrations of ghrelin based on their study that showed ghrelin administration resulted in an increase in adiposity (Tschöp *et al.* 2001a). However, contrary to their hypothesis, they reported that obese Caucasians ($35 \pm 7\%$ body fat) and Pima Indians ($35 \pm 5\%$ body fat) exhibited lower concentrations than their age-matched lean controls suggesting that ghrelin is downregulated in human obesity (Tschöp *et al.* 2001b). The present study would support the latter contention by Tschöp *et al.* (2001) since elephant seal pups are approximately 46% body fat and exhibit circulating ghrelin concentrations that are almost an order of magnitude lower than obese humans (Tschöp *et al.* 2001b).

Increased oxidation of fat in northern elephant seal pups is the primary factor contributing to the animal's metabolism during the postweaning fast, however the endocrine mechanisms regulating fat and carbohydrate metabolism during this period are not well defined. The positive correlation among cortisol, GH and NEFA suggests that cortisol and GH act synergistically to promote lipolysis. Further, the negative correlation between GH and BUN suggests that GH may play a role in decreasing protein catabolism by maintaining lean tissue. Despite the increase in GH, IGF-I was reduced during the fast suggesting that elephant seal pups are GH resistant, possibly mediated by a decrease in hepatic GH receptor expression. The decrease in IGF-I may also alleviate the inhibition on GHRH and GH release resulting in the observed increase in GH. The increase in GH does not appear to be mediated by the increase in ghrelin. However, the increases in ghrelin and glucagon suggest that these hormones may play an integral role in the regulation of glucose metabolism. Despite the increase in ghrelin, concentrations were still almost an order of magnitude lower than those in obese humans supporting the contention of Tschöp *et al.* (2001b) that obesity downregulates ghrelin expression. Because ghrelin has been shown to stimulate food intake, the increase in ghrelin may be an important hormonal signal to the pups to end the fast and initiate feeding at sea. Where our previous studies on thyroid hormones and leptin (Ortiz *et al.* 2001a,b, 2003) provided little information on elucidating the hormonal regulation of fasting metabolism in elephant seal pups, the present study has identified a number of hormones, namely cortisol, GH, ghrelin and glucagon, that may contribute significantly to the regulation of fat, protein and

carbohydrate metabolism during the postweaning fast in northern elephant seal pups.

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References

- Adams S & Costa DP 1993 Water conservation and protein metabolism in northern elephant seal pups during the postweaning fast. *Journal of Comparative Physiology B* **163** 367–373.
- Ahima RS & Flier JS 2000 Leptin. *Annual Review of Physiology* **62** 413–437.
- Bergendahl M, Vance ML, Iranmanesh A, Thorner MO & Veldhuis JD 1996 Fasting as a metabolic stress paradigm selectively amplifies cortisol secretory burst mass and delays the time of maximal nyctohemeral cortisol concentrations in healthy men. *Journal of Clinical Endocrinology and Metabolism* **81** 692–699.
- Boyle PJ, Shah SD & Cryer PE 1989 Insulin, glucagon, and catecholamines in prevention of hypoglycemia during fasting. *American Journal of Physiology* **256** E651–E661.
- Broggio F, Arvat E, Benso A, Gottero C, Muccioli G, Papotti M, van der Lely AJ, Deghenghi R & Ghigo E 2001 Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. *Journal of Clinical Endocrinology and Metabolism* **86** 5083–5086.
- Castellini MA, Costa DP & Huntley AC 1987 Fatty acid metabolism in fasting northern elephant seal pups. *Journal of Comparative Physiology B* **157** 445–449.
- Considine RV & Caro JF 1997 Leptin and the regulation of body weight. *International Journal of Biochemistry and Cellular Biology* **29** 1255–1272.
- Costa DP & Ortiz CL 1982 Blood chemistry homeostasis during prolonged fasting in the northern elephant seal. *American Journal of Physiology* **242** R591–R595.
- Fain JN, Kovacev VP & Scow RO 1965 Effect of growth hormone and dexamethasone on lipolysis and metabolism in isolated fat cells of the rat. *Journal of Biological Chemistry* **240** 3522–3529.
- Fielder PJ & Talamantes F 1987 The lipolytic effects of mouse placental lactogen II, mouse prolactin, and mouse growth hormone on adipose tissue from virgin and pregnant mice. *Endocrinology* **121** 493–497.
- Goodman HM 1968 Multiple effects of growth hormone on lipolysis. *Endocrinology* **83** 300–308.
- Houser DS & Costa DP 2001 Protein catabolism in suckling and fasting northern elephant seal pups (*Mirounga angustirostris*). *Journal of Comparative Physiology B* **171** 635–642.

- Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H & Wakabayashi I 2001 Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and agouti-related protein mRNA levels and body weight in rats. *Diabetes* **50** 2438–2443.
- Keith EO & Ortiz CL 1989 Glucose kinetics in neonatal elephant seals during postweaning aphagia. *Marine Mammal Science* **5** 99–115.
- Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H & Kangawa K 1999 Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* **402** 656–660.
- Kopchick JJ & Andry JM 2000 Growth hormone (GH), GH receptor, and signal transduction. *Molecular Genetics and Metabolism* **71** 293–314.
- Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K & Matsukura S 2001 A role for ghrelin in the central regulation of feeding. *Nature* **409** 194–198.
- Norrelund H, Møller N, Nair KS, Christiansen JS & Jørgensen JOL 2001 Continuation of growth hormone (GH) substitution during fasting in GH-deficient patients decreases urea excretion and conserves protein synthesis. *Journal of Clinical Endocrinology and Metabolism* **86** 3120–3129.
- Ortiz CL, Costa D & Le Boeuf BJ 1978 Water and energy flux in elephant seal pups fasting under natural conditions. *Physiological Zoology* **51** 166–178.
- Ortiz RM, Noren DP, Litz B & Ortiz CL 2001a A new perspective on adiposity in a naturally obese mammal. *American Journal of Physiology, Endocrinology and Metabolism* **281** E1347–E1351.
- Ortiz RM, Wade CE & Ortiz CL 2001b Effects of prolonged fasting on plasma cortisol and TH in postweaned northern elephant seal pups. *American Journal of Physiology* **280** R790–R795.
- Ortiz RM, Houser DS, Wade CE & Ortiz CL 2003 Hormonal changes associated with the transition between nursing and natural fasting in northern elephant seals (*Mirounga angustirostris*). *General and Comparative Endocrinology* **130** 78–83.
- Pernia SD, Hill A & Ortiz CL 1980 Urea turnover during prolonged fasting in the northern elephant seal. *Comparative Biochemistry and Physiology* **65B** 731–734.
- Rea LD & Costa DP 1992 Changes in standard metabolism during long-term fasting in northern elephant seal pups (*Mirounga angustirostris*). *Physiological Zoology* **65** 97–111.
- Salomon F, Cuneo RC, Hesp R & Sönksen PH 1989 The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. *New England Journal of Medicine* **321** 1797–1803.
- Seoane LM, Tovar S, Baldelli R, Arvat E, Ghigo E, Casanueva FF & Dieguez C 2000 Ghrelin elicits a marked stimulatory effect on GH secretion in freely-moving rats. *European Journal of Endocrinology* **143** R7–R9.
- Straus DS 1994 Nutritional regulation of hormones and growth factors that control mammalian growth. *FASEB Journal* **8** 6–12.
- Straus DS & Takemoto CD 1990 Effect of fasting on insulin-like growth factor-I (IGF-I) and growth hormone receptor mRNA levels and IGF-I gene transcription in rat liver. *Molecular Endocrinology* **4** 91–100.
- Takaya K, Ariyasu H, Kanamoto N, Iwakura H, Yoshimoto A, Harada M, Mori K, Komatsu Y, Usui T, Shimatsu A, Ogawa Y, Hosoda K, Akamizu T, Kojima M, Kangawa K & Nakao K 2000 Ghrelin strongly stimulates growth hormone (GH) release in humans. *Journal of Clinical Endocrinology and Metabolism* **85** 4908–4911.
- Thissen J-P, Ketelslegers J-M & Underwood LE 1994 Nutritional regulation of the insulin-like growth factors. *Endocrine Reviews* **15** 80–101.
- Toshinai K, Mondal MS, Nakazato M, Date Y, Murakami N, Kojima M, Kangawa K & Matsujura S 2001 Upregulation of ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. *Biochemical and Biophysical Research Communications* **281** 1220–1225.
- Tschöp M, Smiley DL & Heiman ML 2001a Ghrelin induces adiposity in rodents. *Nature* **407** 908–913.
- Tschöp M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E & Heiman ML 2001b Circulating ghrelin levels are decreased in human obesity. *Diabetes* **50** 707–709.
- Vance ML, Hartman ML & Thorner MO 1992 Growth hormone and nutrition. *Hormone Research* **38** (Suppl 1) 85–88.
- Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DGA, Ghatei MA & Bloom SR 2000 The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* **141** 4325–4328.

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