SEX DIFFERENCES IN FUEL USE AND METABOLISM DURING DEVELOPMENT IN FASTING JUVENILE NORTHERN ELEPHANT SEALS (*MIROUNGA ANGUSTIROSTRIS*)

by

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ABSTRACT

Many polygynous, capital breeders exhibit sexual dimorphism with respect to body size and composition. This dimorphism is often facilitated by sex differences in foraging behavior, growth rates and patterns of nutrient deposition during development. In species that undergo extended fasts during development, metabolic strategies for fuel use have the potential to influence future reproductive success by directly impacting somatic growth and acquisition of traits required for successful breeding. My aim was to investigate sexual dimorphism associated with the metabolic strategies for fasting in developing northern elephant seals. Thirty one juvenile seals of both sexes were sampled over extended fasts during autumn haul-outs. Field metabolic rate (FMR) and protein catabolism were estimated from changes in mass and body composition over 22.6 ± 4.6 (SD)days of fasting. Protein catabolism was assessed directly in a subset of animals based on urea flux at the beginning and end of the fast. Regulatory hormones and blood metabolites measured included growth hormone (GH), cortisol, insulin, glucagon, testosterone, estradiol, glucose, urea and β-hydroxybutyrate. Males exhibited higher rates of energy expenditure during the fast but spared body protein stores more effectively than females. Rates of protein catabolism were strongly influenced by GH levels which varied between the sexes. Estimates of protein catabolism from urea flux were significantly lower than those from mass loss. These data suggest that sex differences in fuel metabolism and energy expenditure during fasting arise early in juvenile development. These differences may play an important role in the development of adult traits associated with reproductive success.

Key-words: dimorphism, fasting, body composition, urea flux, field metabolic rate, seals

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Introduction

In animals which are capital breeders, the energy used for reproduction comes from body reserves. Body reserves available for a given breeding attempt are influenced by lifetime patterns of growth and use during previous fasts. Many capital breeders also use polygynous mating systems with the potential for reproductive success and optimal body composition varying between the sexes. Males may require large size and muscle mass to effectively compete for access to mates while females may require significant adipose tissue reserves to provide the nutrients necessary for milk synthesis. This sexual selection commonly results in sexual dimorphism (Darwin, 1874). Sexual dimorphism is associated with differences in foraging strategies (Ginnett and Demment, 1997; Le Boeuf et al., 2000) growth rates (Setchell et al., 2001) and body compositions (Berry and Shine, 1980; Schulte-Hostedde et al., 2001). Adult male and female animals store energy acquired from foraging differently so it is possible that the sexes have different metabolic strategies for using stored reserves while undergoing natural fasts. These sex differences may be most crucial during key periods of growth and development.

Numerous species of animals undergo natural fasts during critical periods of their life history (Millar and Hickling, 1990). More rarely, some species experience periods of prolonged fasting throughout development (Beauplet et al., 2003; Beck et al., 2003; Castellini and Rea, 1992; Le Maho, 1977; Nelson et al., 1983; Noren and Mangel, 2004). Fasting during development has the potential to influence growth and survival to reproductive ages, as well as the development of features associated with successful

breeding. Juveniles have the greatest energetic demands for growth as they develop toward maturity (Boyd, 2002; Clutton-Brock, 1988; Costa and Williams, 1999; Lindström, 1999).. The duration of the fast and the depletion of body reserves are influenced by the management of fuel reserves. Metabolic strategies for fasting are thus crucial for species where growth and body size influence future reproductive success (Crocker et al., 2001) and directly influence proportional depletion of reserves of body fat and muscle.

Pinnipeds provide numerous examples of sexually dimorphic species that fast during development. Studies on sex differences in fasting metabolism in developing pinnipeds have yielded equivocal results. Arnould et al. (2001) found that female Antarctic fur seal (*Arctocephalus gazelle*; hereafter AFS) pups had greater proportional adipose reserves and higher field metabolic rates (FMR) than males. Despite these differences males and females met equivalent proportions of FMR with protein catabolism. Similarly, a different population of AFS exhibited higher rates of mass loss and FMR in fasting female pups than fasting male pups (Guinet et al., 1999). In contrast a study on sympatric subantarctic fur seals (SAFS), with the longest intersuckling fasts found in otariids, reported no sex differences in FMR but found that leaner males spared protein much more effectively than females (Beauplet et al., 2003). A more recent study on the same population suggested that male and female pups rapidly converged on an identical and low level of protein catabolism and found no sex differences in any aspect of fasting metabolism (Verrier et al., 2009).

Among the pinnipeds, the longest developmental fasts are found in phocid seals. Elephant seal pups can fast for up to 3 months before their first foraging migration (Reiter et al., 1981). Prior to reaching breeding ages, juvenile elephant seals haul out twice a year for approximately one month, during which they fast completely from food and water (Field et al., 2005). Northern elephant seals are capital breeders and exhibit extreme sexual dimorphism with males as large as ten times the mass of females (Deutsch et al., 1994). This size difference is directly related to male growth rates and patterns. Two important features of the growth pattern of male elephant seals are the peak in growth rate which coincides with the onset of puberty at 3- 5 years old and the end of growth upon reaching sexual maturity at age 8 (Clinton, 1990). Quite differently, female elephant seals reach maturity at 4 years old and do not experience a similar growth spurt (Sydeman and Nur, 1994). Clinton and Le Boeuf (1993) suggest that male growth patterns may be associated with sexual selection for large body size in males and the acceleration in growth rate around puberty may be connected to delayed maturity.

Several factors may influence different needs for proportional reserves of fat and muscle in elephant seals. In addition to serving as an energy depot, blubber reserves are important as insulation in the marine environment. This insulation may be particularly important for smaller juveniles with higher surface to volume ratios. Similarly, the larger body size of older males may reduce the need for proportional insulation relative to that of females. Large muscle mass may be critical to the success of males in competitive dominance interactions that are required to breed successfully. These interactions are frequently decided by combat and success is not driven solely by body mass (Haley et al.,

1994). In contrast smaller females need to mobilize significant reserves of lipids to produce the energy dense milk associated with abbreviated periods of parental investment (Crocker et al., 2001). For these reasons females may be selected to prioritize growth of adipose tissue reserves during development to breeding age.

Previous elephant seal studies have yielded highly variable estimates of protein catabolism depending on methodology and life history stage. Studies using urine collection or urea turnover report averages of 1-3% energy from protein catabolism in weaned pups (Adams and Costa, 1993; Houser and Costa, 2001; Pernia et al., 1980) . In contrast, use of mass balance approaches has yielded estimates of protein catabolism contributions to energy metabolism in excess of 15% in individual weaned pups and juveniles (Carlini et al., 2001; Field et al., 2005; Noren et al., 2003). During the post-weaning fast, comparisons of protein catabolism among sexes suggest no differences in fuel use (Carlini et al., 2001; Noren et al., 2003), but there is strong evidence for enhanced protein sparing in male juvenile southern elephant seals (Field et al., 2005). Estimates of protein catabolism in breeding adult animals were slightly higher in females then in males (10% vs. 7% of FMR) (Crocker et al., 1998; Crocker et al., 2001)., The large disparity between estimates of protein catabolism using the two methodologies suggests that mass balance may overestimate protein catabolism relative to direct measures of urea clearance. Additionally failure to account for protein loss as pelage during the molt may increase estimates of protein catabolism during this period (Carlini et al., 2001; Field et al., 2005)

I examined field metabolic rate, body composition, protein catabolism, blood metabolites and regulatory hormones for evidence of sex differences in metabolic strategies for fasting. I investigated sex differences in metabolism and fuel use over the autumn juvenile northern elephant seal haul-out to avoid potential errors associated with molting pelage loss. A second objective was to simultaneously compare estimates of protein catabolism in fasting animals derived from the mass balance method with direct tracer measurements of urea flux.

Methods

Study site and subjects

This study was carried out at Año Nuevo State Reserve, San Mateo County, CA during the autumn juvenile haul out (August-November) in 2008 and 2009. Daily censuses of the rookery were carried out to estimate arrival dates of the animals. Known age 1.8 year old seals were identified and marked with hair dye (Lady Clairol, Stamford, CT) upon arrival to the rookery to facilitate identification throughout the study period. Early fasting samples were collected within six days of arrival to the rookery. An average of 22.6±4.6 (SD) days later animals were recaptured and sampled once more. Body composition was measured using the isotopic dilution method (Iverson et al., 1993). A total of 41 seals were sampled early in the fasting period, 18 males and 22 females. Of these seals, 31 were recovered late in the fasting period, 15 males and 16 females, to complete the measurements. In twelve of these animals protein catabolism was measured by urea flux early in the fasting period and repeated in nine of these seals late in the fasting period for a total of 20 urea flux measurements.

Mass and body composition measurements

Seals were immobilized using an initial intramuscular injection of telazol (teletamine/zolazepam HCl) at a dose of 1.0 mg kg^{-1} and administered intravenous doses of 100 mg ketamine as needed to maintain immobilization (all drugs from Fort Dodge Labs, Fort Dodge IA,USA). Animals were weighed on a canvas tarpaulin connected to a hand winch and a digital scale (MSI tension dynamometer, ±1.0 kg) suspended from an aluminum tripod. A preinjection blood sample was collected via the extradural vein and a bolus injection of 37.0 MBq of tritiated water (HTO) was administered in 2 ml of sterile injectable water. Serial blood sampling demonstrates that HTO equilibration in the total body pool occurs within 90 minutes of intravenous injection in northern elephant seal pups (Houser and Costa, 2001). To confirm equilibration time in juveniles, serial blood samples were collected at 30, 45, 60, 75, 90 and 100 minutes post injection in a subset of animals. All animals were equilibrated by 90 minutes. Equilibration was confirmed for all measurements by comparing two blood samples taken at least 10 minutes apart after 90 minutes. Blood samples were collected in chilled vacutainers, stored on ice, transported to the laboratory and centrifuged for 20 minutes at 2,000 rpm and 4°C. Serum and plasma samples were immediately frozen at -80°C for later analysis. This procedure was repeated late in the fasting period, 22.6±4.6 (SD) days, using a bolus injection of 11.1 MBq of HTO in three ml of sterile water to re-measure the TBW pool.

Water was collected from aliquots of serum (~250 μl) into scintillation vials by way of dry-ice distillation (Ortiz et al 1978). Betaphase scintillation cocktail (7ml,

Westchem, San Diego, CA) was added to each scintillation vial and the specific activity of each sample determined using a Beckmann model LS 6500 liquid scintillation counter (Beckmann, Orange County, California). All samples were analyzed in triplicate. The absolute amount of tracer injected was determined by gravimetric calibration of the syringes used for isotope administration.

Total body water (TBW) was calculated as the total amount of radioactivity injected divided by the radioactivity of the post-equilibration sample. The activity of preinjection blood samples collected during the second sampling period was subtracted from equilibration values in order to account for residual tritium activity from body water measurements made early in the fasting period. TBW determinations were decreased by four percent as the tritium dilution method slightly overestimates TBW volume (Nagy and Costa, 1980; Reilly and Fedak, 1991)

Body composition was calculated from measurements of total body water (TBW) estimated by the tritium dilution method (Iverson et al., 1993), assuming that lipid has no free water and that fat-free mass has a hydration state of 73.3% free water (Iverson et al., 1993; Worthy et al., 1992). Lipid mass was calculated as:

$$M_{lipid} = (M_{total}) - 1.37 * (TBW)$$

where M_{lipid} is lipid mass and M_{total} is total body mass. Lean mass was calculated as the difference between total and lipid mass.

Field metabolic rate (FMR) was estimated using the mass balance method (Crocker et al., 2001; Noren et al., 2003). The amount of energy obtained from lipids was calculated as the changes in lipid mass over the duration of the fast * 39.33 kJ g^{-1}. Lean mass loss was assumed to be 73 % water and 27 % protein and the amount of energy obtained from protein was calculated as 17.99 kJ g^{-1} protein. The sum of energy provided from the changes in lipid mass and protein was then divided by the days elapsed between measurements to estimate the daily field metabolic rate of the animal.

Urea Flux Measurements

In a subset of 12 seals the dilution of ^{14}C-urea was used to calculate urea pool size and rate of flux. Clearance of urea tracer was used to independently estimate the level of protein use throughout the fasting period (Crocker et al 1998; Houser and Costa 2001). (Crocker et al., 1998; Houser and Costa, 2001). An initial blood sample was taken via the extradural vein and a bolus injection of 7.4 MBq of ^{14}C-urea in a four milliliter volume was made into the dorsal extradural vein. Beginning at 30 minutes, blood samples were taken every 15 minutes for the next 90 minutes to confirm equilibration of the isotope in the urea pool (Crocker et al., 1998). Seals were briefly recaptured 1.9 ± 0.64 (SD) days later and a blood sample taken to measure clearance of the labeled urea. Nine animals were recaptured 21.7 ± 2.8(SD) days after the initial procedure and the entire procedure repeated.

The ^{14}C- activity of the serum was determined in triplicate. Two-hundred micro-liter samples were combined with seven milliliters of Betaphase scintillation cocktail and

counted with a Beckman model LS 6500 liquid scintillation counter (Beckmann, Orange County, California).

The pool volume was calculated from the dilution of the injectate at equilibrium. Urea pool size was calculated from the dilution volume and blood urea nitrogen (BUN) concentration. Mean urea flux was calculated based on the flux constant derived from ^{14}C flux curves and a measure of the urea pool size. This urea flux constant (K_u) was calculated as:

$$K_u = \Delta \ln (A) * t^{-1},$$

where A is the activity of the sample and t is time in days. Thus, urea flux constant is the negative slope of a semilog plot of the clearance curve. Urea pool size (N_u) in grams was calculated as:

$$N_u = [BUN]_{(t-0)} * DPM_i/A_{(t-0)},$$

where DPM_i is the total injected activity of ^{14}C, $A_{(t-0)}$ is the equilibration activity, and $[BUN]_{(t-0)}$ is serum urea concentration at equilibrium. Assuming amino acids are the only significant source of nitrogen for urea formation, the daily rate of protein catabolism (r_p) was calculated as:

$$r_p = r_u * 2.292 \text{ g protein g}^{-1} \text{ urea},$$

where r_u is the mean urea flux rate. Energy derived from, protein catabolism was calculated as 17.99 kJ g^{-1} protein (Costa, 1987). Percent energy from protein catabolism was estimated as the proportion of energy from protein relative to FMR.

Metabolite and Hormone Analysis

Serum samples drawn prior to tracer injections were used in measurement of cortisol, insulin, growth hormone (GH), testosterone, and estradiol. Plasma samples drawn prior to tracer injections were used in measurement of glucagon, glucose, β-hydroxybutyrate (β-HBA) and blood urea nitrogen (BUN). Cortisol, insulin, glucagon, total testosterone, estradiol, and GH were analyzed using commercially available RIA Kits (All Siemens, except GH, Millipore). All kits have been previously validated for use in elephant seals (Ortiz et al. 2003a, 2003b, Champagne et al. 2005, 2006). The mean intra-assay coefficient of variation was 3.1, 2.3, 2.1, 5.8, 1.9, and 1.9 % for cortisol, insulin, glucagon, GH, testosterone and estradiol, respectively.

Plasma glucose was measured in duplicate using an YSI 2300 glucose autoanalyzer (YSI, Yellow Springs, OH). β-HBA was measured in duplicate using colorimetric assay (Cayman Chemical Co, Ann Arbor, MI). BUN was measured in duplicate using an enzymatic colorimetric assay (Stanbio labs, Boerne, TX).

Statistical Analysis

Changes across the fast were evaluated using linear mixed effects models with individual seal as a random effect subject term (SAS 9.2). The initial model that was run for all animals included sex, fasting status and their interaction.. This interaction term was removed from the model if not significant ($p \geq 0.05$). Relationships between variables within fasting periods or across the sampling period were evaluated using simple linear

regression. Model residuals were evaluated to assess approximate normality. Means are presented as ± 1 SD.

Results

Mass and body composition

Initial body mass did not vary significantly between the sexes ($F_{1,30}$ = 0.47, p = 0.50; Table 1). Seals lost 13.9 ± 3.6% of body mass over the measurement period and this value did not vary between the sexes ($F_{1,30}$ = 0.47, p = 0.50; Table 1). Initial body composition did not vary between the sexes ($F_{1,29}$ = 0.92, p = 0.3). However, males depleted greater proportions of initial body fat than females (19.6 ± 5.9% vs. 14.5 ± 3.7%; $F_{1,29}$ = 7.45, p < 0.05; Table 1).

Field metabolic rate and energy from protein

When controlling for mass, FMR was greater in males than in females ($F_{2,28}$ = 7.2, p < 0.05; Fig. 1, Table 1). Females obtained a greater percentage of FMR from protein than males ($F_{2,28}$ = 11.0, p < 0.01; Table 1). However, the absolute rate of protein loss was only marginally different ($F_{2,28}$ = 3.00, p = 0.07). Initial body fat proportion was not a significant predictor of the percentage of FMR obtained from protein ($F_{1,29}$ = 0.02, p = 0.90; Fig. 2).

Metabolites

Sexes did not vary in plasma glucose ($F_{1,30}$ = 0.18, p = 0.67, Table 2), β-HBA ($F_{1,29}$ = 0.06, p = 0.80, Table 2) or BUN ($F_{1,30}$ = 0.01, p = 0.91, Table 2) concentrations.

There was no change in the concentration of glucose ($F_{1,30} = 0.40$, $p = 0.53$, Table 2) or β-HBA ($F_{1,29} = 0.02$, $p = 0.88$, Table 2) across the fasting period Mean BUN concentrations decreased significantly across the fasting period ($F_{1,30} = 99.2$, $p < .0001$, Table 2). With one exception, there were no significant relationships between any of the measured hormones and measured metabolites in the early or late samples ($p > 0.05$). In the early fast sample of females, plasma β-HBA concentrations were effected by estradiol levels (Fig. 3).

Hormones

Neither glucagon ($F_{1,30} = 0.02$, $p = 0.88$, Table 3) nor insulin ($F_{1,30} = 2.71$, $p = 0.11$; Table 3) concentrations varied significantly between the sexes. Glucagon increased 34% between the early (41.7 pg mL^{-1}) and late (55.78 pg mL^{-1}) sampling periods ($F_{1,30} = 7.13$, $p = 0.01$; Table 3); however, insulin did not change across the measurement period ($F_{1,30} = 1.15$, $p = 0.29$) and remained relatively low (Table 3). Insulin to glucagon molar ratio (I/G) did not decrease significantly across the fasting period ($F_{1,30} = 0.01$, $p = 0.91$; Table 3). There were no sex differences detected in cortisol concentrations ($F_{1,29} = 0.06$, $p = 0.80$; Table 3). Cortisol concentrations did not change across the fasting period ($F_{1,29} = 0.44$, $p = 0.51$; Table 3).

GH concentrations varied between the sexes ($F_{1,29} = 10.5$, $p < 0.01$; Table 4). GH concentrations decreased significantly across the fast ($F_{1,29} = 24.0$, $p < 0.0001$; Table 4). Male and female GH concentrations declined 68 and 36 % respectively, indicating a significant sex difference in the way GH changes across the fasting period ($F_{1,29} = 24.0$, p

< 0.0001; Table 4). No significant changes across the fast were detected in the testosterone concentrations in the male animals ($F_{1,14} = 0.5$, $p = 0.48$; Table 4). Initial testosterone levels in males decreased with initial body fat (Fig. 4). Female estradiol concentrations declined significantly across the fasting period ($F_{1,15} = 5.1$, $p < 0.05$; Table 4).

Hormones, metabolites and protein loss

Mean BUN was a not a significant predictor of the percentage of FMR obtained from protein catabolism ($F_{1,29} = 0.48$, $p = 0.49$) or the absolute rate of protein loss ($F_{1,29} = 0.51$, $p = 0.48$) . The percentage of FMR obtained from protein catabolism varied negatively with mean GH concentrations over the fast (Fig. 5). The percentage FMR obtained from protein catabolism varied negatively with average estradiol concentrations in females ($F_{1,14} = 6.55$, $p < 0.05$).

In order to look for synergistic or additive effects of hormones I ran models that included average, early or late GH, cortisol and sex hormone (testosterone for males and estradiol for females) concentrations and looked for effects on the proportion of FMR met by protein catabolism. This analysis yielded different results for the sexes. In females, average hormone values accounted for 53% of the variation in protein catabolism, with GH and estradiol having significant effects ($p = 0.04, 0.03$). Early fast blood samples gave a similar result with the hormones accounting for 51% of the variation in proportional protein catabolism. In contrast for late samples only cortisol significantly affected protein catabolism ($p < 0.01$) predicting 51% of the variation in

protein catabolism (Fig. 6). In males, only hormone concentrations late in the fast were significant predictors of protein catabolism. Together late values of GH, testosterone and cortisol accounted for 51% of the variation in protein catabolism with all three hormones having significant effects ($p = 0.01, 0.04, 0.03$).

Urea flux

Urea flux rate decreased significantly across the fasting period ($F_{1,7} = 47.6$, $p < 0.001$; Table 5). While the ability to directly compare the two methods was limited due to differing sample intervals, I compared the early, late and the average of the early and late rates of protein loss estimated from urea flux measurements to those estimated from mass loss. The rate of protein loss was similar when comparing the early fast urea flux measurements to those from mass loss over the fast ($t = 1.2$, $df = 8$, $p = 0.3$). However, the rate of protein loss was significantly lower in the average ($t = 3.01$, $df = 7$, $p < 0.05$) and final ($t = 6.06$, $df = 7$, $p < 0.05$) urea flux measurements when compared to those from mass loss.

Discussion

My data suggest several important sex differences in metabolism, substrate utilization and hormonal regulation of fasting metabolism in juvenile elephant seals. It is notable that these differences are present prior to the rapid growth spurt and onset of dimorphic traits in males. Although there were no sex differences detected in mass or body composition at the start of the fast, notable differences were found in the way stored energy was used during the fast. While juvenile elephant seals spend a considerable

portion of the haul-out sleeping, they also spend a substantial amount of time swimming, terrestrially locomoting and in agonistic interactions with conspecifics. Males spend considerable time in mock dominance interactions that mimic the adult breeding behaviors. Energy expenditure for male and females respectively averaged 2.3 and 2.0 times the predicted standard metabolic rate (SMR) as predicted from Kleiber's equation, or a 15% higher FMR in males. While I made no attempt to quantify behavior in this study, this increase may reflect different activity budgets for males or intrinsically higher resting metabolic rates.

Animals which have greater adipose reserves are generally able to spare protein more efficiently while fasting. This relationship is thought to be driven by a differential ability to mobilize lipid stores and has been reported in a wide variety of species (Goodman et al., 1980) including lactating female northern elephant seals (Crocker et al., 2001) and weaned pups (Noren et al., 2003). However, my results show that males spared lean tissue more effectively than females despite the fact that both sexes had similar adipose reserves at the beginning of the fast. The lack of relationship between adipose reserves and protein sparing in juvenile animals is unexpected and suggests sex differences in regulation of fuel utilization that are independent of effects of the depot available for mobilization.

Similar to the findings in weanling northern elephant seals (Houser et al., 2001), urea flux, rates of protein loss and BUN declined across the fast in juveniles. In contrast, BUN does not change across the fasting period in adult northern elephant seals (Crocker

et al., 1998). In lactating adults, increases in glomerular filtration rate (GFR) late in lactation uncoupled static BUN values from rates of urea flux in adult females, so that BUN declined as urea flux increased. In contrast, weaned pups maintained a more consistent GFR across the fast (Houser et al., 2001) so that BUN values better reflected reductions in urea flux across the fast. In the current study BUN was not correlated with rates of urea flux. This suggests potential variation in GFR in fasting juveniles and provides further evidence that standard clinical proxies for metabolite flux need to be interpreted with caution in wildlife systems.

Most puzzling in the current study was the decline in GH across the fast in both sexes in association with reduced rates of protein loss. Thus, despite evidence that increased suppression of GH levels negatively influence rates of protein catabolism in individuals, suppression of mean GH levels across the fast were associated with reduced protein loss. Growth hormone increases in response to fasting in most species and is important in the conservation of protein during fasting (Eigenmann and de Bruijne, 1985; Ho et al., 1988; Webster et al., 1999). The metabolic effects of GH include increased lipolysis and stimulation of IGF-I activity, which promotes reuptake of mobilized amino acids by muscle (Nørrelund et al., 2001). Juvenile male seals have significantly higher GH concentrations than females, suppress GH levels less across the fast and have lower contributions of protein catabolism to FMR. Taken together these data suggest that other hormones may also be important in regulating the level of protein catabolism across the fast and that this regulation varies between the sexes.

In females, higher estradiol levels at the beginning of the fast were associated with higher ketone levels, a proxy for rates of lipid oxidation. Morrow et al. (1981a) reported that doses of estradiol and progesterone administered to female rats accelerated ketogenesis during fasting. In a subsequent study the same group found that chronic exposure to oral birth control pills in pre-menopausal women promotes an amplification of ketone bodies in the blood early in the fasting period (Morrow et al., 1981b). In both studies the effect of sex steroids on ketosis is limited to the initial part of the fast and this relationship is no longer detected later in the fasting period. Likewise, the females in the present study did not demonstrate a relationship between β-HBA and estradiol late in the fasting period. A more recent study attributes the underlying cause for sexual dimorphism found in lipid metabolism to the discovery that many genes are expressed in a sexually dimorphic way, suggesting differences in post-translational changes in human men and women; very likely resulting in different enzyme activities, abundance of cellular signal transduction elements and substrate kinetics (Mittendorfer, 2005).

Females with higher early fast GH levels had lower rates of protein catabolism over the fast. Across the fast, estradiol and GH levels declined and values late in the fast had no impact on rates of protein catabolism. In contrast cortisol levels were maintained and levels late in the fast were strongly related to rates of protein catabolism. This is consistent with the well established impact of cortisol on protein mobilization and catabolism (Brillon et al., 1995). A completely different pattern of regulation was suggested in the males. Hormone levels early in the fast had no influence on rates of protein catabolism but values late in the fast were important. So males that had higher

testosterone and GH levels at the end of the fast were better at suppressing protein catabolism over the preceding several weeks. Cortisol levels late in the fast had the opposite effect on protein catabolism as that seen in females, with higher cortisol associated with reduced protein use. This suggests that the positive effects of cortisol on lipid mobilization dominate over impacts on protein mobilization when GH and testosterone levels are sufficient to promote reuptake of mobilized amino acids into muscle. This is consistent with evidence that GH prevents protein catabolic effects of glucocorticoids (Horber and Haymond, 1990). Testosterone has been shown to interact positively with GH to regulate energy expenditure, fat metabolism and protein anabolism by modifying GH responsiveness (Yu et al., 1996).

Glucose homeostasis while fasting is predominantly regulated by insulin and glucagon interactions in birds and mammals. Additionally, increased substrate mobilization and stimulation of gluconeogenesis are correlated with declining I/G ratios (Cuendet et al., 1975; Hazelwood, 1984). Investigations in adult males (D. Crocker, *unpublished data*) and weanling elephant seals show declining I/G ratios while fasting which result from increased glucagon levels and stable insulin levels (Champagne et al., 2005). Juvenile elephant seals show a similar pattern to adult males and weanlings with increasing glucagon levels and stable insulin levels. Lactating, Female elephant seals reduced I/G ratios via suppression of insulin alone which also led to declining I/G ratios across the fasting period (Champagne et al., 2006; Houser et al., 2007). In all age classes the reduction of I/G ratio was associated with maintenance of glucose levels across the fast. In contrast, there was no significant change in the juvenile I/G ratios across the

fasting period. Plasma glucagon concentrations were low compared to fasting adapted birds (Cherel et al., 1988) and non-fasting adapted mammals (Unger and Orci, 1976) and were less than or similar to that recorded in other fasting adapted mammals.

Previous studies proposed that low insulin levels in fasting-adapted species are a crucial adaptation to promote high levels of lipid mobilization during fasting (Mustonen et al., 2004). Insulin acts on adipocytes, blocking lipolyis and fat mobilization via inhibition of intracellular triglyceride hydrolization. Lipogenesis is favored in the presence of insulin via facilitation of hepatic fatty acid synthesis from glucose and uptake of glucose for glycerol formation in adipocytes. In contrast, Cortisol and GH are known to inhibit lipogenesis and/or stimulate lipolysis (Djurhuus et al., 2004). In the present study insulin and cortisol did not change during the fasting period and GH decreased significantly. Similar findings were reported in breeding adult male elephant seals (D. Crocker, *unpublished data*). However, studies in weanling pups and lactating females revealed dramatic increases in cortisol (Champagne et al., 2005; Champagne et al., 2006) and GH across the fasting period (Ortiz et al., 2003). These differences may reflect the metabolic constraints of mobilization of fat and protein for milk synthesis during lactation or protein mobilization for rapid synthesis of respiratory pigments during the post-weaning fast. Without these constraints, fasting animals may not need to increase substrate mobilization across the fast through elevation of cortisol and GH when compared to conspecifics. However, these patterns still represent a significant diversion from the typical regulation of metabolism associated with fasting. Despite these differences my data provide strong evidence that levels of these hormones directly

influence protein sparing during the fast and provide a potential mechanism underlying sex differences in this feature.

For most species, extended fasting will markedly increase the concentration of β-HBA. However, despite a predominantly fat based metabolism the accumulation of this ketone body was negligible in this study. This suggests perhaps elevated rates of ketoacid usage which are independent of plasma levels or curtailed ketogenesis. No change was found in the levels of β-HBA for either sex and the concentration remained exceptionally low when compared to other fasting adapted species (Cherel and Le, 1988; LeBlanc et al., 2001). The direct opposite was found in breeding adult males and females and weanling northern elephant seals with β-HBA increasing significantly across the fast in all three age classes (Champagne et al., 2005; Champagne et al., 2006; Crocker, *unpublished data*). Juvenile levels were similar to the early fasting measurements made in breeding adults and considerably lower than weanlings. The average fasting duration for adults and weanlings was up to twice as long as the period in juveniles. This shorter juvenile fasting period could contribute to the lack of change detected and it is possible that sampling later in the fast would have shown the same increasing trend found in adults and weanlings.

Although measured over different time periods, my study allowed some comparison of protein catabolism estimates derived from the mass balance method with direct tracer measurements of urea flux. The two methods gave significantly different estimates of the rate of protein catabolism and the percentage of energy derived from

protein. As seen in other studies, here the mass balance method slightly overestimates the use of protein when compared to urea flux. Despite the fact that this study demonstrates a reasonable agreement in the estimates from both methods (i.e. low rates of protein catabolism), the values determined from the mass balance method were significantly lower than other estimates published for elephant seals (Carlini et al., 2001; Field et al., 2005; Noren et al., 2003). One potential explanation for some of these differences is the error associated with pelage loss in molting periods. The water isotope dilution method for determining body composition assumes a consistent hydration state for fat free mass. Individual or fasting variation in this parameter may reduce the precision in estimates of protein catabolism from mass balance. Alternatively, the urea flux method assumes all nitrogenous waste from protein catabolism results in urea production. Significant loss of urinary nitrogen as ammonia or uric acid would result in underestimates of protein catabolism. Adams and Costa (1993) examined urinary nitrogen output in elephant seal weanlings and found that ~9% of urinary nitrogen was in the form of ammonia or uric acid. This would result in a commensurate underestimate of protein loss. The average of the two urea flux estimates or r_p was ~36% lower than that from mass loss. However, it is unknown over what time course the measured suppression in urea flux occurred making direct comparisons problematic. Both methods resulted in low estimates of the contribution of protein to metabolism (< 5%) that seems reasonable in a fasting adapted species.

In summary, juvenile northern elephant seals exhibited sex differences in metabolic strategies for fasting including differences in FMR, the contribution of protein

catabolism to FMR and hormonal regulation of fasting. More effective protein sparing in males during biannual fasts throughout development may contribute to body composition differences in adults that are associated with breeding success. Conversely, maintenance of higher adipose tissue stores may be crucial to preparing for the eventual mobilization of lipids for synthesis of energy dense milk in primiparous females. My findings suggest that sex differences arise early in development, prior to visual evidence of sexual dimorphism, and that for species that undergo frequent extended natural fasts, sex differences in metabolism may contribute to the development of dimorphic adult phenotypes.

References

Adams, S. H. and Costa, D. P. (1993). Water conservation and protein metabolism in northern elephant seal pups during the postweaning fast. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* 163, 367-373.

Arnould, J. P. Y., Green, J. A. and Rawlins, D. R. (2001). Fasting metabolism in Antarctic fur seal (Arctocephalus gazella) pups. *Comparative Biochemistry and Physiology-Part A: Molecular & Integrative Physiology* 129, 829-841.

Beauplet, G., Guinet, C. and Arnould, J. P. Y. (2003). Body composition changes, metabolic fuel use, and energy expenditure during extended fasting in subantarctic fur seal (Arctocephalus tropicalis) pups at Amsterdam Island. *Physiological and Biochemical Zoology* 76, 262-270.

Beck, C. A., Bowen, W. D. and Iverson, S. J. (2003). Sex differences in the seasonal patterns of energy storage and expenditure in a phocid seal. *Journal of Animal Ecology* 72, 280-291.

Berry, J. F. and Shine, R. (1980). Sexual size dimorphism and sexual selection in turtles (Order Testudines). *Oecologia* 44, 185-191.

Boyd, I. (2002). Energetics: consequences for fitness. *Marine Mammal Biology–an Evolutionary Approach*, 247–277.

Brillon, D. J., Zheng, B., Campbell, R. G. and Matthews, D. E. (1995). Effect of cortisol on energy expenditure and amino acid metabolism in humans. *American Journal of Physiology- Endocrinology And Metabolism* 268, E501.

Carlini, A. R., Márquez, M. E. I., Ramdohr, S., Bornemann, H., Panarello, H. O. and Daneri, G. A. (2001). Postweaning duration and body composition changes in southern elephant seal (Mirounga leonina) pups at King George Island. *Physiological and Biochemical Zoology* 74, 531-540.

Castellini, M. A. and Rea, L. D. (1992). The biochemistry of natural fasting at its limits. *Cellular and Molecular Life Sciences* 48, 575-582.

Champagne, C. D., Houser, D. S. and Crocker, D. E. (2005). Glucose production and substrate cycle activity in a fasting adapted animal, the northern elephant seal. *Journal of experimental biology* 208, 859-868.

Champagne, C. D., Houser, D. S. and Crocker, D. E. (2006). Glucose metabolism during lactation in a fasting animal, the northern elephant seal. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 291, R1129.

Cherel, Y. and Le, M. (1988). Changes in body mass and plasma metabolites during short-term fasting in the king penguin. *The condor* 90, 257-258.

Cherel, Y., Robin, J. P., Walch, O., Karmann, H., Netchitailo, P. and Le Maho, Y. (1988). Fasting in king penguin. I. Hormonal and metabolic changes during breeding. *American Journal of Physiology- Regulatory, Integrative and Comparative Physiology* 254, 170.

Clinton, W. L. (1990). Sexual-selection and the life-history of male northern elephant seals: University of California, Santa Cruz.

Clinton, W. L. and Le Boeuf, B. J. (1993). Sexual selection's effects on male life history and the pattern of male mortality. *Ecology* 74, 1884-1892.

Clutton-Brock, T. H. (1988). Reproductive success: University of Chicago Press Chicago.

Costa, D. P. (1987). 4 Isotopic Methods For Quantifying Material and Energy Intake of Free-Ranging Marine Mammals. *Approaches to marine mammal energetics*, 43.

Costa, D. P. and Williams, T. M. (1999). Marine mammal energetics. *Biology of marine mammals. Smithsonian Institution Press, Washington, DC*, 176–217.

Crocker, D. E., Webb, P. M., Costa, D. P. and Le Boeuf, B. J. (1998). Protein catabolism and renal function in lactating northern elephant seals. *Physiological Zoology* 71, 485-491.

Crocker, D. E., Williams, J. D., Costa, D. P. and Le Boeuf, B. J. (2001). Maternal traits and reproductive effort in northern elephant seals. *Ecology* 82, 3541-3555.

Cuendet, G. S., Loten, E. G., Cameron, D. P., Renold, A. E. and Marliss, E. B. (1975). Hormone-substrate responses to total fasting in lean and obese mice. *American Journal of Physiology* 228, 276.

Darwin, C. (1874). The Descent of Man, and Selection in Relation to Sex. 1871. *London: Murray*, 135.

Deutsch, C., Le Boeuf, B. J. and Costa, D. P. (1994). Sex differences in reproductive effort in northern elephant seals. *Elephant seals: population ecology, behavior, and physiology. University of California Press, Berkeley, California, USA*, 169–210.

Djurhuus, C. B., Gravholt, C. H., Nielsen, S., Pedersen, S. B., Moller, N. and Schmitz, O. (2004). Additive effects of cortisol and growth hormone on regional and systemic lipolysis in humans. *American Journal of Physiology- Endocrinology And Metabolism* 286, E488.

Eigenmann, J. E. and de Bruijne, J. (1985). Insulin-like growth factor I and growth hormone in canine starvation. *European Journal of Endocrinology* 108, 161.

Field, I. C., Bradshaw, C. J. A., Burton, H. and Hindell, M. A. (2005). Juvenile southern elephant seals exhibit seasonal differences in energetic requirements and use of lipids and protein stores.

Ginnett, T. F. and Demment, M. W. (1997). Sex differences in giraffe foraging behavior at two spatial scales. *Oecologia* 110, 291-300.

Goodman, M. N., Larsen, P. R., Kaplan, M. M., Aoki, T. T., Young, V. R. and Ruderman, N. B. (1980). Starvation in the rat. II. Effect of age and obesity on protein sparing and fuel metabolism. *The American journal of physiology* 239, E277.

Guinet, C., Goldsworthy, S. D. and Robinson, S. (1999). Sex differences in mass loss rate and growth efficiency in Antarctic fur seal (Arctocephalus gazella) pups at Macquarie Island. *Behavioral Ecology and Sociobiology* 46, 157-163.

Haley, M. P., Deutsch, C. J. and Le Boeuf, B. J. (1994). Size, dominance and copulatory success in male northern elephant seals, Mirounga angustirostris. *Animal Behaviour* 48, 1249-1260.

Hazelwood, R. L. (1984). Pancreatic hormones, insulin/glucagon molar ratios, and somatostatin as determinants of avian carbohydrate metabolism. *Journal of Experimental Zoology* 232, 647-652.

Ho, K. Y., Veldhuis, J. D., Johnson, M. L., Furlanetto, R., Evans, W. S., Alberti, K. G. and Thorner, M. O. (1988). Fasting enhances growth hormone secretion and amplifies the complex rhythms of growth hormone secretion in man. *Journal of Clinical Investigation* 81, 968.

Horber, F. F. and Haymond, M. W. (1990). Human growth hormone prevents the protein catabolic side effects of prednisone in humans. *Journal of Clinical Investigation* 86, 265.

Houser, D. and Costa, D. (2001). Protein catabolism in suckling and fasting northern elephant seal pups (Mirounga angustirostris). *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* 171, 635-642.

Houser, D. S., Crocker, D. E., Webb, P. M. and Costa, D. P. (2001). Renal function in suckling and fasting pups of the northern elephant seal. *Comparative Biochemistry and Physiology-Part A: Molecular & Integrative Physiology* 129, 405-415.

Iverson, S. J., Bowen, W. D., Boness, D. J. and Oftedal, O. T. (1993). The effect of maternal size and milk energy output on pup growth in grey seals (Halichoerus grypus). *Physiological Zoology* 66, 61-88.

Le Boeuf, B. J., Crocker, D. E., Costa, D. P., Blackwell, S. B., Webb, P. M. and Houser, D. S. (2000). Foraging ecology of northern elephant seals. *Ecological Monographs* 70, 353-382.

Le Maho, M. (1977). The Emperor Penguin: a strategy to live and breed in the cold. *Am. Sci* 65, 680-693.

LeBlanc, P. J., Obbard, M., Battersby, B. J., Felskie, A. K., Brown, L., Wright, P. A. and Ballantyne, J. S. (2001). Correlations of plasma lipid metabolites with hibernation and

lactation in wild black bears Ursus americanus. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* 171, 327-334.

Lindström, J. (1999). Early development and fitness in birds and mammals. *Trends in Ecology & Evolution* 14, 343-348.

Millar, J. S. and Hickling, G. J. (1990). Fasting endurance and the evolution of mammalian body size. *Functional Ecology* 4, 5-12.

Mittendorfer, B. (2005). Sexual dimorphism in human lipid metabolism. *Journal of Nutrition* 135, 681.

Morrow, P. G., Marshall, W. P., Kim, H. J. and Kalkhoff, R. (1981a). Metabolic response to starvation. I. Relative effects of pregnancy and sex steroid administration in the rat* 1. *Metabolism* 30, 268-273.

Morrow, P. G., Marshall, W. P., Kim, H. J. and Kalkhoff, R. K. (1981b). Metabolic response to starvation. II. Effects of sex steroid administration to pre-and postmenopausal women* 1. *Metabolism* 30, 274-278.

Mustonen, A. M., Nieminen, P., Puukka, M., Asikainen, J., Saarela, S., Karonen, S. L., Kukkonen, J. V. K. and Hyvärinen, H. (2004). Physiological adaptations of the raccoon dog (Nyctereutes procyonoides) to seasonal fasting-fat and nitrogen metabolism and influence of continuous melatonin treatment. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* 174, 1-12.

Nagy, K. A. and Costa, D. P. (1980). Water flux in animals: analysis of potential errors in the tritiated water method. *American Journal of Physiology- Regulatory, Integrative and Comparative Physiology* 238, R454.

Nelson, R. A., Folk Jr, G. E., Pfeiffer, E. W., Craighead, J. J., Jonkel, C. J. and Steiger, D. L. (1983). Behavior, biochemistry, and hibernation in black, grizzly, and polar bears. *Bears: Their Biology and Management*, 284-290.

Noren, D. P., Crocker, D. E., Williams, T. M. and Costa, D. P. (2003). Energy reserve utilization in northern elephant seal (Mirounga angustirostris) pups during the

postweaning fast: size does matter. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* 173, 443-454.

Noren, D. P. and Mangel, M. (2004). Energy reserve allocation in fasting Northern Elephant Seal Pups: inter relationships between body condition and fasting duration. *Functional Ecology* 18, 233-242.

Nørrelund, H., Nair, K. S., Jørgensen, J. O. L., Christiansen, J. S. and Møller, N. (2001). The protein-retaining effects of growth hormone during fasting involve inhibition of muscle-protein breakdown. *Diabetes* 50, 96.

Ortiz, R. M., Noren, D. P., Ortiz, C. L. and Talamantes, F. (2003). GH and ghrelin increase with fasting in a naturally adapted species, the northern elephant seal (Mirounga angustirostris). *Journal of Endocrinology* 178, 533.

Pernia, S. D., Hill, A. and Leo Ortiz, C. (1980). Urea turnover during prolonged fasting in the northern elephant seal. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 65, 731-734.

Reilly, J. J. and Fedak, M. A. (1991). Rates of water turnover and energy expenditure of free living male common seals (Phoca vitulina). *Journal of Zoology* 223, 461-468.

Reiter, J., Panken, K. J. and Le Boeuf, B. J. (1981). Female competition and reproductive success in northern elephant seals. *Animal Behaviour* 29, 670-687.

Schulte-Hostedde, A. I., Millar, J. S. and Hickling, G. J. (2001). Sexual dimorphism in body composition of small mammals. *Canadian Journal of Zoology* 79, 1016-1020.

Setchell, J. M., Lee, P. C., Wickings, E. J. and Dixson, A. F. (2001). Growth and ontogeny of sexual size dimorphism in the mandrill (Mandrillus sphinx). *American Journal of Physical Anthropology* 115, 349-360.

Sydeman, W. J. and Nur, N. (1994). Life history strategies of female northern elephant seals. *Elephant seals. Population ecology, behavior, and physiology*, 137-153.

Unger, R. H. and Orci, L. (1976). Physiology and pathophysiology of glucagon. *Physiological Reviews* 56, 778.

Verrier, D., Groscolas, R., Guinet, C. and Arnould, J. P. Y. (2009). Physiological response to extreme fasting in subantarctic fur seal (Arctocephalus tropicalis) pups: metabolic rates, energy reserve utilization, and water fluxes. *American Journal of Physiology- Regulatory, Integrative and Comparative Physiology* 297, R1582.

Webster, J. R., Corson, I. D., Littlejohn, R. P., Stuart, S. K. and Suttie, J. M. (1999). Effects of Photoperiod on the Cessation of Growth during Autumn in Male Red Deer and Growth Hormone and Insulin-like Growth Factor-I Secretion* 1. *General and Comparative Endocrinology* 113, 464-477.

Worthy, G. A. J., Morris, P. A., Costa, D. P. and Le Boeuf, B. J. (1992). Moult energetics of the northern elephant seal (Mirounga angustirostris). *Journal of Zoology* 227, 257-265.

Yu, Y. M., Domene, H. M., Sztein, J., Counts, D. R. and Cassorla, F. (1996). Developmental changes and differential regulation by testosterone and estradiol of growth hormone receptor expression in the rabbit. *European Journal of Endocrinology* 135, 583.

Table 1: Mean (SD) of initial body mass, body composition, percentage loss, daily rate of protein loss (r_p) field metabolic rate (FMR), and proportion of FMR from protein catabolism which were measured early and late in the fasting period. Variables which are significantly different between the sexes are marked by similar superscripts ($p < .05$)

Sex	Initial mass (kg)	Mass loss (%)	Initial fat (%)	Fat loss (%)	r_p (g/day)	FMR (MJ day^{-1})	% FMR
Male	189.4 (24.8)	14.3 (3.4)	35.1 (1.2)	19.6 (5.9)[1]	68.8 (20.0)	34.1 (5.4)[2]	3.6 (1.3)[3]
Female	160.4 (22.6)	13.5 (3.9)	35.1 (2.0)	14.5 (3.7)[1]	84.1 (28.2)	30.6 (5.9)[2]	5.2 (2.0)[3]
All	187.3 (23.2)	13.9 (3.6)	35.0 (1.6)	17.0 (15.0)	76.7 (25.4)	32.3 (5.9)	4.5 (1.9)

Table 2: Mean (SD) of blood urea nitrogen, glucose and β – HBA which were measured early and late in the fasting period. Significantly different values are marked by similar superscripts. (Linear mixed effects model $p < .05$)

Sex	**Initial BUN** ($mg\ dl^{-1}$)	**Final BUN** ($mg\ dl^{-1}$)	**Initial glucose** ($mg\ dl^{-1}$)	**Final glucose** ($mg\ dl^{-1}$)	**Initial β–HBA** (mM)	**Final β–HBA** (mM)
Male	26.2 (6.9)[1]	18.0 (4.3)[1]	137.1 (15.0)	137.0 (19.0)	279.1(224.0)	225.1 (110.0)
Female	27.0 (5.0)[2]	16.2 (3.7)[2]	137.2±13.0	134.8 (9.4)	256.6 (110.0)	257.8 (78.6)
All	26.5 (5.8)[3]	17.1 (4.1)[3]	41.7(16.0)	55.8 (32.6)	255.7 (170.2)	251.7 (92.6)

Table 3: Mean (SD) of cortisol, insulin, glucagon, and I:G which were measured early and late in the fasting period. Significantly different values are marked by similar superscripts. (Linear mixed effects model $p < .05$).

Sex	Initial cortisol ($\mu g\ dl^{-1}$)	Final cortisol ($\mu g\ dl^{-1}$)	Initial insulin ($pg\ ml^{-1}$)	Final insulin ($pg\ ml^{-1}$)	Initial glucagon ($pg\ ml^{-1}$)	Final glucagon ($pg\ ml^{-1}$)	Initial I:G	Final I:G
Male	7.9 (5.7)	9.9 (6.1)	82.3 (44.4)	101.7 (71.5)	42.8 (19.6)	53.4 (37.0)	1.4 (0.9)	1.4 (1.2)
Female	9.0 (7.3)	8.9 (6.6)	90.4 (43.9)	102.5 (37.2)	40.9[1] (13.0)	58.0[1] (28.9)	1.6 (1.4)	1.5 (1.4)
All	8.1 (6.0)	9.4 (6.3)	86.8 (43.7)	102.1 (55.5)	41.7[2] (16.0)	55.8[2] (32.6)	1.5 (1.2)	1.5 (1.3)

Table 4: Mean (SD) of GH, testosterone and estradiol which were measured early and late in the fasting period. Significantly different values are marked by similar superscripts. ($p < 0.05$). nd = none detected

Sex	Initial GH (ng ml^{-1})	Final GH (ng ml^{-1})	Initial testosterone (ng ml^{-1})	Final testosterone (ng ml^{-1})	Intial estradiol (pg ml^{-1})	Final estradiol (pg ml^{-1})
Male	4.7^{1} (2.6)	1.5^{2} (0.9)	45.3 (26.9)	40.3 (21.1)	nd	nd
Female	2.2^{1} (1.7)	1.4^{2} (0.6)	nd	nd	105.6^{4} (22.3)	82.0^{4} (37.0)
All	3.3^{3} (2.5)	1.4^{3} (0.8)	45.3 (26.9)	40.3 (21.1)	105.6 (22.3)	82.0 (37.0)

Table 5: Urea flux constant (k), urea flux (r_u) and rate of protein loss (r_p) per day determined from the urea flux method. Significantly different means are marked with similar subscripts. (student's t-test p = 0.0002)

Seal ID	Initial k	Final k	Initial r_p (g/day)	Final r_p (g/day)	Initial r_u (g/day)	Final r_u (g/day)
♀U544	0.89	0.86	66.0	46.6	28.8	20.3
♀U883	0.98	0.68	88.5	27.5	38.6	12.0
♀4019	0.84	0.79	55.8	22.0	24.3	9.6
♀4021	0.73	0.46	50.0	22.8	21.8	9.9
♀X109	0.87	0.73	82.8	36.2	36.1	15.8
♂T850	1.02	0.83	87.9	43.1	38.3	18.8
♂U35	0.82	0.67	60.6	39.8	26.4	17.4
♂4020	0.72	0.70	54.3	25.5	23.7	11.1
♂X545	0.83	----	49.3	----	21.5	----
♂U244	0.74	----	64.5	----	28.1	----
♀4016	0.91	----	50.6	----	22.1	----
♂V621	----	0.91	----	50.6	----	22.1
Mean	**0.85 (0.10)**	**0.74 (0.10)**	**64.6[1] (15.1)**	**34.9[1] (10.8)**	**28.1[2] (6.6)**	**15.2[2] (4.7)**

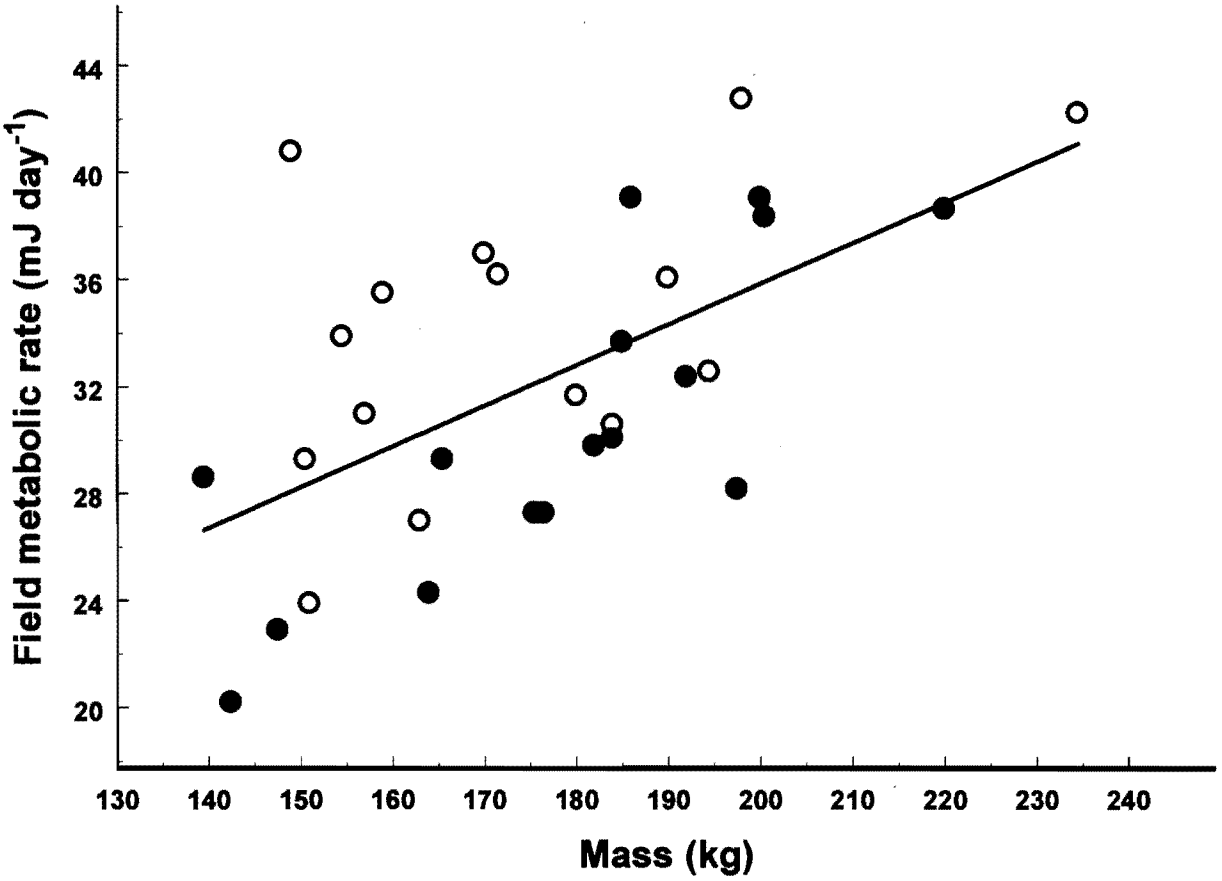

Figure 1: Relationship between average body mass and field metabolic rate (MJ day^{-1}). The equation for the regression line is $y = 5.385244 + 0.152493x$ ($r^2 = .35$, $F_{1,29} = 15.61$, $p < .0001$) Field metabolic rate calculated based on the mass balance method. Closed circles represent females, open circles males.

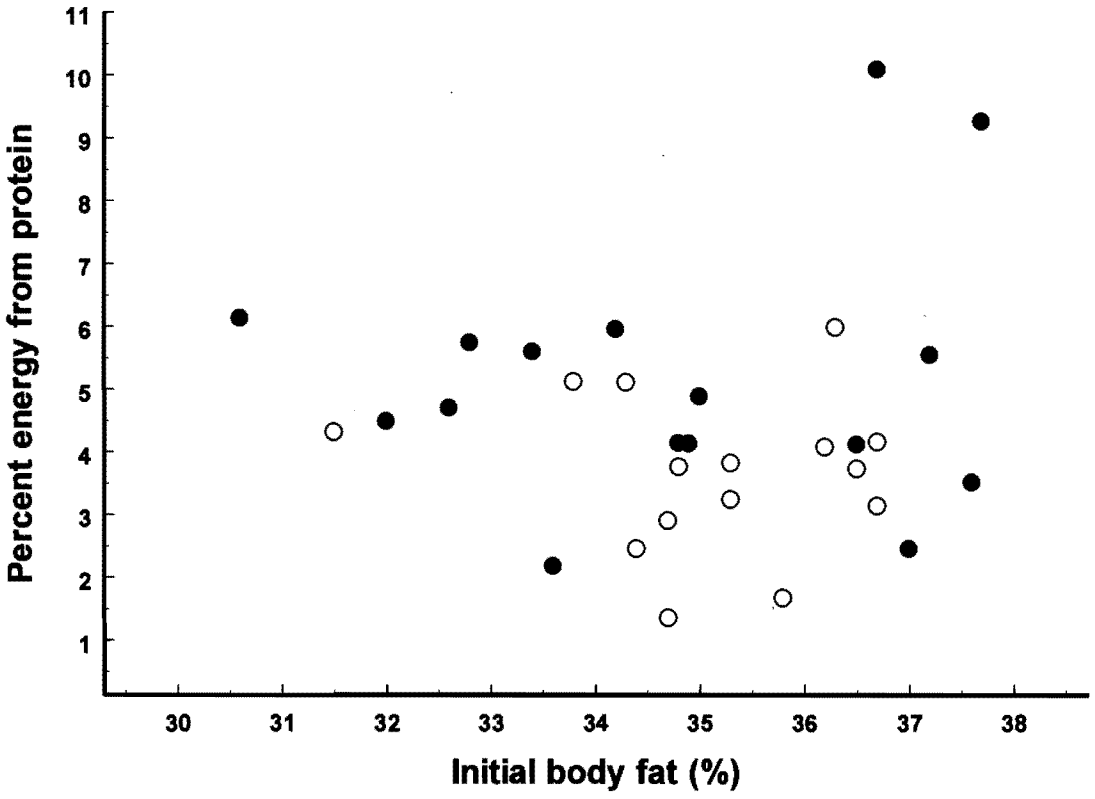

Figure 2: Relationship between initial body composition and percent energy from protein derived from mass balance method. Closed circles are females, open circles males.

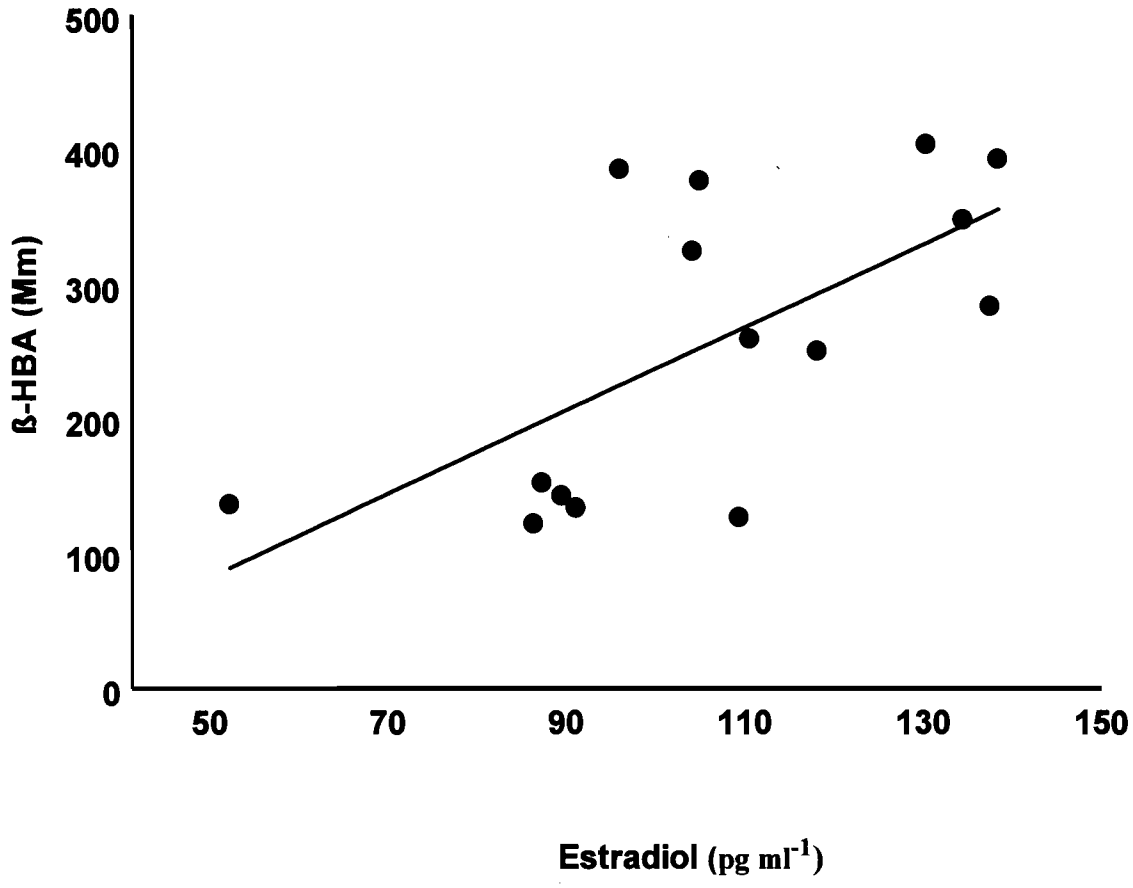

Figure 3: Relationship between initial female estradiol and β-HBA concentrations . The equation for the regression line is $y = -72.5 + 3.10x$ ($r^2 = 0.43$, $F_{1,13} = 9.9$, $p = 0.007$)

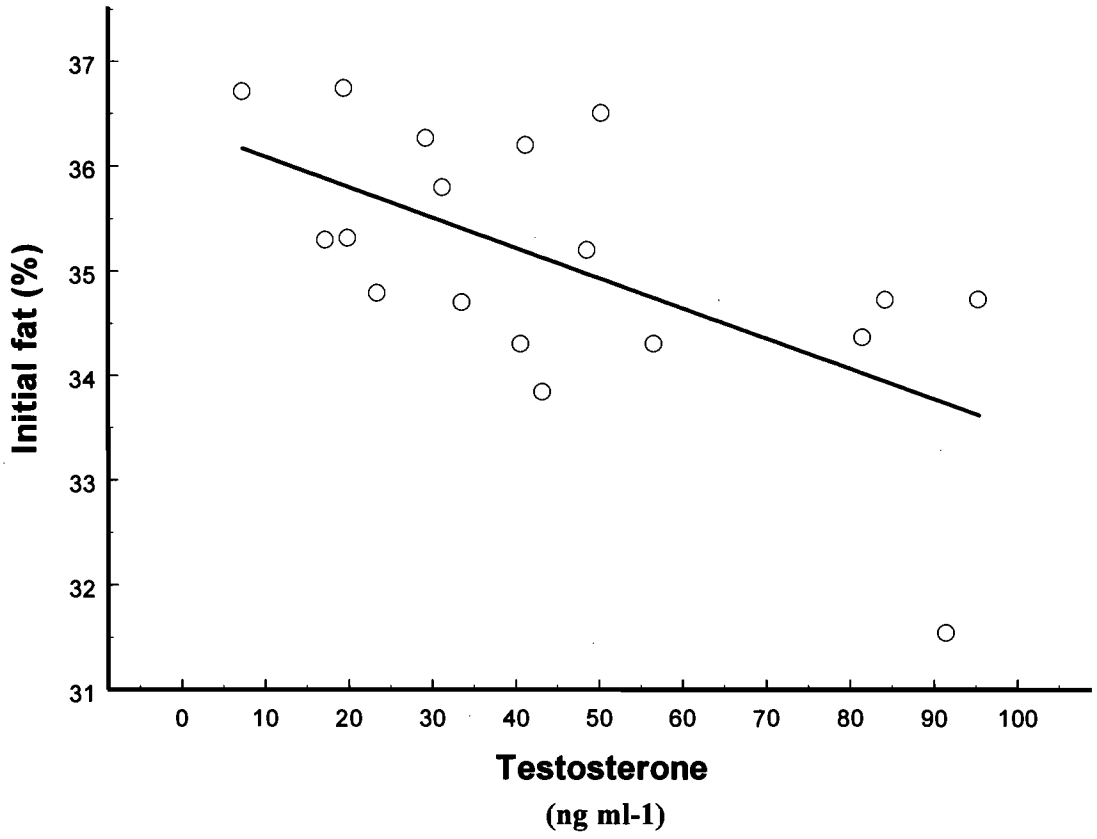

Figure 4: Relationship between male testosterone and initial body fat. The equation for the regression line is $y = 0.4 - 2.9 \times 10^{-4} x$ ($r^2 = 0.38$, $F_{1,16} = 9.8$, $p < 0.05$).

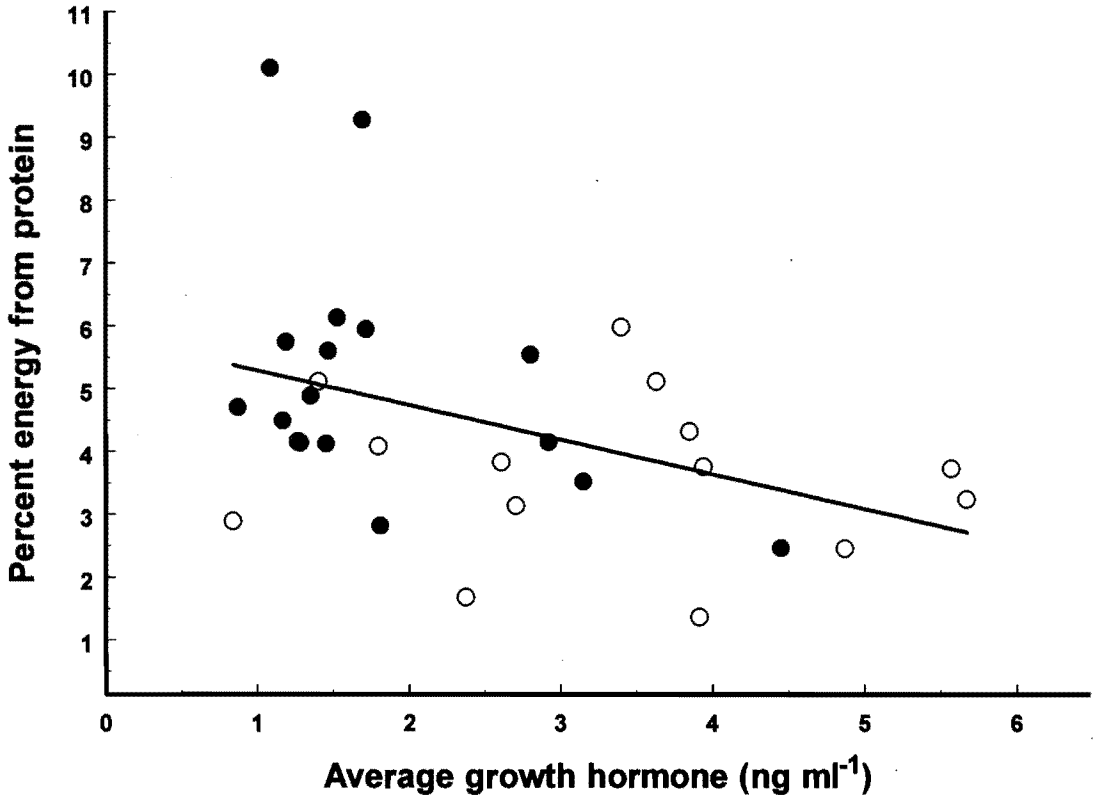

Figure 5: Relationship between average growth hormone and percent energy from protein. The equation for the regression line is $y = 5.8 - 0.55x$. ($r^2 = 0.17$, $F_{1, 29} = 6.05$, $p = 0.02$). Open circles represent males, closed circles represent females.

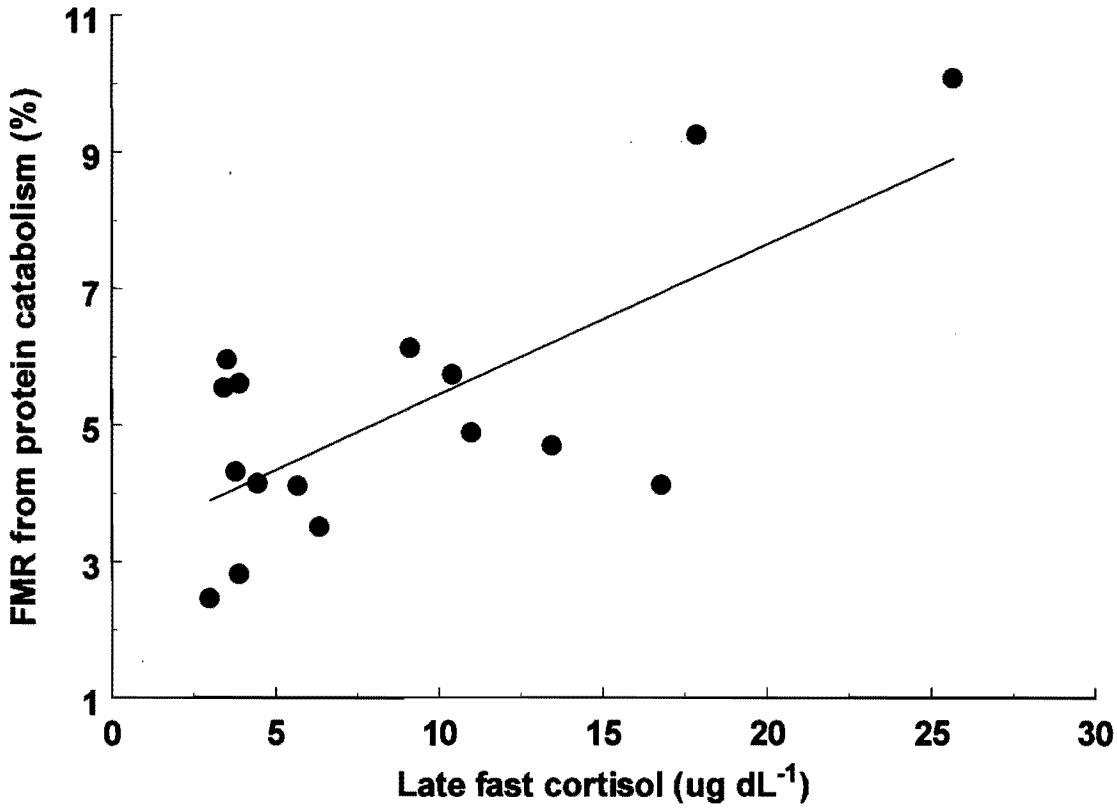

Figure 6. Changes in the contribution of protein catabolism to FMR with late fast cortisol levels in female juvenile seals ($y= 3.23 + 0.22x$; $r^2 = 0.51$, $F_{1,14} = 14.6$, $p < 0.01$).