Hormonal changes associated with the transition between nursing and natural fasting in northern elephant seals (Mirounga angustirostris)

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Abstract

To better interpret previously described hormonal changes observed during the natural postweaning fast (2–3 months) endured by pups of the northern elephant seal (Mirounga angustirostris), we compared plasma cortisol, thyroid hormones, and leptin in pups (n = 5) measured during nursing and fasting periods. Blood samples were taken at four times; early (9 days postpartum) and late (18–22 days postpartum) nursing, and early (second week postweaning) and late (eighth week postweaning) fasting. Plasma cortisol increased 39% between early and late nursing and almost 4-fold by late fasting. After the early nursing period, cortisol and body mass were negatively correlated (y = 28.3 – 0.19x; R = 0.569; p = 0.027). Total thyroxine (tT4), free T4 (fT4), total triiodothyronine (tT3) and reverse T3 (rT3) were greatest at early nursing and reduced by late nursing and remained so throughout the fast, with the exception of tT4, which increased between late nursing (17.7 ± 2.1 ng mL–1) and late fasting (30.1 ± 2.8 ng mL–1) periods. Leptin remained unaltered among the four sampling periods and was not correlated with body mass. Pups appear to exhibit a shift in the relationship between cortisol and body mass suggesting a potential role for cortisol in the regulation of body fat. The higher concentrations of tT3 and tT4 during early nursing may reflect enhanced growth and development during this period, however the increase late in fasting is likely physiologically insignificant and an artifact of reduced metabolic clearance of these hormones. Transition of the pups from nursing to fasting states is characterized by a striking lack of change in cortisol, thyroid hormones, and leptin suggesting that any metabolic alterations associated with this transition may occur independent of these hormones.

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1. Introduction

Northern elephant seal (Mirounga angustirostris) (NES) pups nurse for approximately one month during which time they increase their body fat stores to approximately 45–50% of body mass (Houser and Costa, 2001; Ortiz et al., 1978; Rea and Costa, 1992). After nursing, pups fast for 2–3 months as a natural component of their life history. The extensive fat stores the pups accrue during the nursing period provide the primary source of energy during the postweaning fast, supporting >90% of their metabolic rate (Houser and Costa, 2001; Rea and Costa, 1992). The transition between nursing and fasting is associated with a significant change in body fat and fat metabolism (Rea and Costa, 1992). Relationships between fasting duration and plasma cortisol, thyroid hormones, and leptin have been previously examined (Houser et al., 2001; Ortiz et al., 2001a,b). However, these hormones have yet to be examined in pups as they transition between a nursing and fasting state.

In fasting NES pups we previously observed a linear increase in circulating cortisol and slight, however significant, increases in plasma total thyroxine (tT4) and triiodothyronine (tT3) (Ortiz et al., 2001b). Leptin remained relatively low and unaltered. Although leptin did not display a relationship with body fat, cortisol was negatively correlated with fat mass (Ortiz et al., 2001a). These previously described hormonal changes suggest...
that (1) cortisol may be involved in the regulation of body fat, (2) the kinetics of thyroid hormone metabolism and clearance are altered during the fast, and (3) leptin does not appear to regulate the metabolism of body fat as in terrestrial mammals (Ortiz et al., 2001a,b). An examination of these hormones in nursing pups would provide perspective by which fasting concentrations could be more accurately interpreted.

Therefore, the present study quantified changes in plasma cortisol, thyroid hormones, and leptin as pups transitioned between nursing and fasting. Because the transition between nursing and fasting is associated with a significant change in body fat and fat metabolism, this study contributes to elucidating the endocrine regulation of fasting energetics in a species adapted to prolonged fasting. Furthermore, an examination of the hormonal changes in NES pups as they transition between nursing and fasting provides an opportunity to augment the findings of previous studies in fasting pups.

2. Materials and methods

2.1. Animals and sampling

Details of animal handling and blood sampling have been described previously (Houser and Costa, 2001; Houser et al., 2001). In brief, pregnant adult females were monitored daily to determine parturition dates. Within two days of birth, adult females were marked with hair dye (Lady Clairol, Stamford, CT) and pups (three males, two females) were marked with bleach. Blood samples used in the present study were used in conjunction with previous studies on the energetics of nursing and fasting pups (Houser and Costa, 2001; Houser et al., 2001). Pups were sampled at 9 (early nursing) and 18–22 days postpartum (late nursing) and during the second (early fasting) and eighth (late fasting) week of their post-weaning fast. Pups during the nursing period were separated from their mothers for blood sampling and weighing without chemical immobilization. Separation of mother-pups and the subsequent procedures did not appear to compromise the mother-pup relationship as mothers did not demonstrate any rejection behaviors upon reunion of the two, all pups exhibited growth rates (4.4kgdays\(^{-1}\)) similar to those reported for other NES pups (Rea and Costa, 1992), and all pups were weaned within the normal period for NES pups (approximately 27 days, (Le Boeuf et al., 1972)). Fasting, weaned pups were sedated with 1mgkg\(^{-1}\) of tiletamine-zolazepam (Telazol, Fort Dodge, IA) for blood sampling and weighing. After blood sampling, body mass was measured by placing each pup in a nylon restraint bag and suspending it from a tripod with a hanging-load cell scale, which had a capacity of 455 kg with an error of ±2.3 kg. Blood samples were collected in a 5-ml Na-heparin vacutainer, gently agitated, and placed on ice until they were returned to the lab for centrifugation and collection of plasma, which was usually within 1 h of collection.

2.2. Analyses

Cortisol, leptin, tT4, fT4, tT3, and reverse T3 (rT3) were measured by commercially available radioimmunoassays and thyroid stimulating hormone (TSH) was measured by immunoradiometric assay. Cortisol, leptin, tT4, fT4, and tT3 have been previously validated for NES plasma (Houser et al., 2001; Ortiz et al., 2001a,b). TSH (DPC, Los Angeles, CA) and rT3 (ALPCO Diagnostics, Windham, NH) were validated for NES plasma in the present study. Serially diluted samples displayed significant parallelism with the standard curve for TSH and rT3. All samples were analyzed in the same assay with intra-assay coefficient of variability between 5 and 10%.

2.3. Statistics

Means (±SE) were analyzed by two-way analysis of variance adjusted for repeated measures over time (early and late nursing, and early and late fasting). Fisher’s PLSD was administered post hoc if significance group × time interaction was observed at p < 0.05. Simple regressions were used to examine the correlations between two variables during the nursing and fasting periods. Slopes of the regressions during both nutritional states were compared by analysis of covariance (ANCOVA). If slopes were not significantly different, then data were combined to produce a single regression. If slopes were significantly different, then data for the two periods were not combined and treated separately. Statistics were conducted using Statview software (SAS, 1998).

3. Results

Body mass was increased 2.4-fold between early and late nursing periods and reduced 18% between early and late fasting (Table 1). Body mass and cortisol were not significantly correlated during the nursing period; however, when data from the late nursing period were included in the correlation with the fasting data, cortisol was negatively and significantly correlated with body mass (cortisol = 28.3 – 0.19 body mass; R = 0.569; p = 0.027) (Fig. 1A). Because body mass and cortisol were not different between the late nursing and early fasting periods, the data for the late nursing period was included in the regression with the fasting data. Cortisol increased 39% between early and late nursing and 4-fold
Fig. 2. Changes in mean (A) plasma total T3 (\(tT3\); □) and reverse T3 (\(rT3\); ○), and (B) plasma total T4 (\(tT4\); ■) and free T4 (\(fT4\); ◆) in five northern elephant seal pups during early (9 days postpartum) and late (18–22 days postpartum) nursing periods and during early (second week postweaning) and late (eighth week postweaning) fasting periods. Means were considered significant at \(p < 0.05\). *Significantly different from all sampling periods. #Significantly different from late nursing and early fasting.

Table 1

<table>
<thead>
<tr>
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<th>Nursing</th>
<th>Fasting</th>
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<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Late</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>48 ± 3(^a)</td>
<td>115 ± 6(^b)</td>
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<tr>
<td>Cortisol ((\mu)g(\cdot)dl(^{-1}))</td>
<td>3.6 ± 0.3(^a)</td>
<td>5.0 ± 0.8(^b)</td>
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<tr>
<td>TSH (ng(\cdot)mL(^{-1}))</td>
<td>1.52 ± 0.09</td>
<td>1.58 ± 0.10</td>
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<tr>
<td>*Leptin (ng(\cdot)mL(^{-1}))</td>
<td>3.5 ± 0.2 (9.0)</td>
<td>4.0 ± 0.3 (12.1)</td>
</tr>
<tr>
<td>(tT3 : tT4) (ng(\cdot)ng(^{-1}))</td>
<td>0.023 ± 0.003(^a)</td>
<td>0.031 ± 0.004(^b)</td>
</tr>
<tr>
<td>(tT3 : rT3) (ng(\cdot)pg(^{-1}))</td>
<td>0.074 ± 0.008(^a)</td>
<td>0.041 ± 0.005(^b)</td>
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Significantly different \((p < 0.05)\) means (±SE) are denoted by uncommon superscript letters.

*Mean values for leptin are reported for \(n = 4\), excluding the one outlier (values in parentheses) (see Section 3 for explanation). TSH = thyroid stimulating hormone.

Fig. 1. Correlations between body mass and (A) plasma cortisol, and (B) plasma total T4 (\(tT4\)) for five northern elephant seal pups during early (9 days postpartum) and late (18–22 days postpartum) nursing periods and during early (second week postweaning) and late (eighth week postweaning) fasting periods. Each symbol represents an individual pup at each sampling period. Samples during the early nursing period were not used to construct the correlation between body mass and cortisol (see Section 3 for explanation). Correlation between \(tT4\) and body mass only represents data during the nursing period because data were not significantly correlated during the fasting period. Correlations were considered significant at \(p < 0.05\).
by late fasting (Table 1). Total T₄ was negatively and significantly correlated with body mass during the nursing period (total T₄ = 75.6 – 0.49 body mass; R = 0.927; p < 0.0001), but the relationship was not significant during the fast (Fig. 1B). Leptin and body mass were not positively correlated during nursing or fasting periods. Because one of the animals exhibited leptin concentrations that were more than two standard deviations above the mean values for each sampling period, this animal’s leptin values were considered outliers and not included in the calculation of means (±SE) presented in Table 1. All thyroid hormones were higher during early nursing than at any of the other measurement periods, and with the exception of tT₄, thyroid hormones did not change during the fast (Fig. 2A and B). Mean tT₄ increased 33% between early and late fasting, but this value remained 57% lower than the mean for early nursing (Fig. 2B). Leptin and TSH remained unaltered throughout the nursing and fasting periods (Table 1). Free and total T₄ (free T₄ = –1.25 + 0.31 total T₄; R = 0.931; p < 0.0001) were positively and significantly correlated during both nursing and fasting periods. Because regression slopes for the relationship between fT₄ and tT₄ during both periods were not different (p = 0.08), data for the nursing and fasting periods were combined to produce a single correlation. Total T₃ and rT₃ were positively and significantly correlated during the fasting period (reverse T₃ = 0.49 + 0.91 total T₃; R = 0.674; p = 0.032), but not during the nursing period.

4. Discussion

Some mammals are adapted to intermittent periods of food deprivation that occur as predictable and repeatable components of their life histories. Prolonged periods of food deprivation are observed in hibernating hedgehogs or wintering bears, for example, and are associated with altered concentrations of circulating glucocorticoids and thyroid hormones (Azizi et al., 1979; Fowler, 1988). The present study reveals that the transition between nursing and fasting is associated with a striking lack of change in cortisol, thyroid hormones and leptin suggesting that physiological changes associated with fasting are initiated prior to fasting and that weaning may not initiate these changes. However, like many hibernators, pups exhibit changes in cortisol and tT₄ over the course of the fast. The hormonal changes during the nursing period provide perspective from which to gauge the changes in cortisol and thyroid hormones observed during the fasting period reported previously (Houser et al., 2001; Ortiz et al., 2001a,b).

Glucocorticoids have been shown to increase in the European ground squirrel (*Citellus citellus*) (Shivatcheva et al., 1988), the hedgehog (*Erinaceus europaeus*) (Saboureau et al., 1980), and the little brown bat (*Myotis lucifugus lucifugus*) (Gustafson and Belt, 1981) during hibernation, a period in which these animals experience prolonged food deprivation similar to that experienced by fasting elephant seals. The increasing trend in glucocorticoids observed in the aforementioned hibernators is consistent with that in fasting pups. However, this increase during hibernation may be the exception as suggested by Gustafson and Belt (1981) (see review by (Hudson and Wang, 1979). As previously suggested for hibernating bats (Gustafson and Belt, 1981), the increase in glucocorticoids may serve an important role in supporting the energetic demands of metabolically active pups with respect to the mobilization of fatty acids and maintenance of gluconeogenesis. It is well documented that the oxidation of fat is the primary source of energy in fasting pups (Castellini et al., 1987; Rea and Costa, 1992) and that circulating glucose is consistently high throughout the fast (Costa and Ortiz, 1982; Ortiz et al., 2001b).

The observed negative correlation between cortisol and body mass is similar to that previously reported given that body mass and fat mass are significantly and positively correlated (Ortiz et al., 2001a). Interestingly, this correlation is not significant until after the early nursing period when plasma cortisol and body mass (and presumably body fat) are the lowest indicating that the shift in the relationship between cortisol and the initial change in body mass does not occur until after appreciable body fat has accrued. This shift in the relationship between cortisol and body mass suggests that body fat and circulating concentrations of cortisol may be closely associated, as discussed previously (Ortiz et al., 2001a,b).

The increase in thyroid hormones observed in hibernating squirrels has been attributed to a decrease in clearance from circulation (Demenex and Henderson, 1978). Fasting NES pups exhibited increases in tT₃, tT₄, and fT₄, attributed to a decrease in clearance from circulation, a decrease in binding of the free fraction, and a decrease in deiodination of T₄ (Ortiz et al., 2001b). Although only tT₄ was elevated late in fasting when compared to early fasting in the present study, tT₃ and fT₄ displayed an increasing trend consistent with our previous study (Ortiz et al., 2001b). Lack of a significant increase may have been a consequence of sample size since our previous study had a sample size of 15 pups (Ortiz et al., 2001b). Regardless, concentrations of these hormones late in the fast pale in comparison to concentrations early in the nursing period suggesting that the observed increases in the present and previous studies (Ortiz et al., 2001b) may be attributed to alterations in hormone metabolism and clearance and not a result of increased production. In contrast, bears, which are closely related to seals (Ray, 1976), exhibit a decrease in thyroid hormones (tT₄, fT₄, and T₃) during
their winter sleep (Azizi et al., 1979). Although higher concentrations of tT3, tT4, and rT3 early in nursing may reflect a period of enhanced neonatal growth and development, they may also be a consequence of thermoregulatory demands during a period when body fat is relatively low (Rea and Costa, 1992). The decrease in thyroid hormones between day 9 and days 18–22 of nursing is consistent with that observed during the postnatal period in other seals including grey (Halichoerus grypus) (Woldstad and Jenssen, 1999), harbor (Phoca vitulina) (Haulena et al., 1998), harp (P. groenlandica) (Stokkan et al., 1995), hooded (Cystophora cristata) (Stokkan et al., 1995), and southern elephant seals (M. leonina) (Little, 1991).

In the European hedgehog, thyroxine concentrations decrease the month prior to the onset of hibernation despite the fact that body mass is maintained or continues to increase suggesting an anticipatory response to hibernation (Fowler, 1988). These concentrations remain suppressed during the first half of hibernation and begin to increase during the second half. The seasonal pattern of thyroxine in the hedgehog is similar to that observed in pups in the present study.

Also, in hibernating hedgehogs, increased thyroxine was associated with a decrease in body mass (Fig. 8; Fowler, 1988); however the increase in thyroxine is thought to be a result of altered secretion and not altered metabolic clearance and to be in anticipation of arousal from hibernation. Although tT3 and body mass during the nursing period also exhibited a significant and negative correlation, a cause-and-effect relationship can not be ascertained from the present study. Nonetheless, during the nursing period tT4 concentrations are reduced as body mass is increased suggesting some relationship between body mass and thyroid hormones may exist. A similar relationship between tT4 and body mass has been reported for grey seal pups (Woldstad and Jenssen, 1999). Because tT4 and body mass were not correlated during the fast, the relationship between tT4 and body mass during the nursing period may reflect a shift in metabolic state as previously suggested for harbour seals (Haulena et al., 1998).

Reduction in circulating T3 concentrations in response to food deprivation in mammals may be an adaptive mechanism by which the metabolic rate is lowered to diminish its demand on body reserves (Burger et al., 1980; Cheikh et al., 1994). The reduction in T3 is usually accompanied by a reciprocal rise in the biologically inactive rT3, which is another mechanism to spare the animal from the oxygen-demanding actions of T3 (Eales, 1988; Suda et al., 1978). Site-specific monodeiodination of T4 at either the 5-position on the tyrosyl (inner) ring or at the S’-position on the phenolic (outer) ring results in either rT3 or T3, respectively (St. Germain, 1994). The positive correlation between tT3 and rT3 during the fasting period indicates that these hormones changed in parallel and suggests that site-specific monodeiodination favoring the conversion of T4 to T3 over T3 was not likely in fasting NES pups. The conversion of T4 to T3 could also have been reduced by the presence of elevated cortisol. In restraint-induced stress rats, elevated glucocorticoids reduced both hepatic and renal 5’ deiodinase activity, which resulted in a decrease in circulating T3 (Bianco et al., 1987). Using the tT3-to-tT4 ratio as an index of conversion in the present study, the ratio was reduced during the fast compared to late nursing suggesting that the conversion of T4 to T3 may have been impaired by the presence of increasing cortisol in fasting pups. If so, then fasting pups may not require increased production of biologically inactive rT3.

Fasting is usually associated with a reduction in body mass and serum leptin concentrations, while during naturally fed conditions leptin concentrations are elevated (Ahima and Flier, 2000). Previously, we described a lack of a change in circulating leptin throughout the fast and a lack of a correlation between leptin and body fat, which led us to suggest that leptin is dissociated from the reductions in body and fat mass during the fast in NES pups and that leptin may not play a role in regulating body fat (Ortiz et al., 2001a,b). The lack of a change in leptin concentrations during the nursing and fasting periods in the present study would support our contention that leptin has a minimal, if any, role in the regulation of body fat in these animals.

In summary, plasma cortisol exhibited a significant increase between nursing and fasting periods in fasting NES pups, and thus, was inversely proportional to body mass following the early nursing period when body fat mass is the lowest suggesting a shift in the response of cortisol to a change in body fat. Body mass was correlated with tT4 during the nursing, but not the fasting period, possibly reflecting a shift in nutritional status as suggested for harbor seals (Haulena et al., 1998). Increases in thyroid hormone concentrations late in the fast pale in comparison to those during the early nursing period suggesting that the clearance and metabolism of these hormones are altered in fasting pups. The lack of a change in leptin between nursing and fasting provides further evidence that leptin may not play a significant role in the regulation of body fat in these animals. However, a condition of chronic hypoleptinemia may be a physiological adaptation which allowed marine mammals to obtain relatively large fat depots in order to inhabit a thermally challenging, aquatic environment. The striking lack of a change in circulating concentrations of cortisol, leptin and thyroid hormones between late nursing and early fasting periods suggests that the metabolic adjustments associated with the transition from a fed to fasting state occur independent of these measured hormones. Also, this lack of hormonal change between late nursing and early fasting periods suggest...
that the physiological adaptations necessary to survive the ensuing 2–3 months fast are initiated well before the inception of the fast, supporting the previous contention that nursing elephant seal pups are pre-adapted to the postweaning fast (Houser and Costa, 2001).

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