Effects of prolonged fasting on plasma cortisol and TH in postweaned northern elephant seal pups

RUDY M. ORTIZ,1,2 CHARLES E. WADE,2 AND C. LEO ORTIZ1
1Department of Biology, University of California, Santa Cruz 95064; and 2Neuroendocrinology Lab, Division of Life Sciences, National Aeronautics and Space Administration Ames Research Center, Moffett Field, California 94035

Received 10 July 2000; accepted in final form 31 October 2000

Ortiz, Rudy M., Charles E. Wade, and C. Leo Ortiz.
Effects of prolonged fasting on plasma cortisol and TH in postweaned northern elephant seal pups. Am J Physiol Regulatory Integrative Comp Physiol 280: R790–R795, 2001.—Northern elephant seal (Mirounga angustirostris) pups rely on the oxidation of fat stores as their primary source of energy during their 8- to 12-wk postweaning fast; however, potential endocrine mechanisms involved with this increased fat metabolism have yet to be examined. Therefore, 15 pups were serially blood sampled in the field during the first 7 wk of their postweaning fast to examine the changes in plasma concentrations of cortisol and thyroid hormones (TH), which are involved in fat metabolism in other mammals. Cortisol increased, indicating that it contributed to an increase in lipolysis. Increased total triiodothyronine (tT3) and thyroxine (T4) may not reflect increased thyroid gland activity, but rather alterations in hormone metabolism. tT3-to-tT4 ratio decreased, suggesting a decrease in thyroxine (T4) deiodination, whereas the negative correlation between total proteins and free T4 suggests that the increase in free hormone is attributed to a decrease in binding globulins. Changes in TH are most similar to those observed during hibernation than starvation in mammals, suggesting that the metabolic adaptations to natural fasting are more similar to hibernation despite the fact these animals remain active throughout the fasting period.

fat metabolism; food deprivation; leptin; marine mammals

NORTHERN ELEPHANT SEAL (Mirounga angustirostris) (NES) pups nurse for ~1 mo before their postweaning fast, which may last as long as 3 mo (4, 23). Pups may increase their body fat to ~50% of body mass while they are nursing (26). During their postweaning fast, these extensive fat stores are the primary source of energy, supporting >95% of their metabolic rate (4, 26). Although such extended periods of complete abstinence from food would induce adverse effects on energy balance in most mammals, NES pups have developed robust physiological mechanisms to withstand this potentially detrimental period.

In terrestrial mammals, extended periods of food restriction are usually associated with an increase in glucocorticoids (3, 28) and a decrease in thyroid hormones (20, 22) and leptin (2). During periods of reduced food intake or fasting, glucocorticoids help to provide energy by increasing lipolysis (3) and help to maintain circulating glucose concentrations via increased gluconeogenesis (10). Glucocorticoids can also lessen the adverse effects of metabolic acidosis incurred during fasting by increasing Na+/K+-H+-ATPase synthesis (32). The increase in fat oxidation previously described in NES pups (4) suggests that glucocorticoids may be elevated during the fast as in other mammals.

Because thyroid hormones are associated with an increase in metabolic rate, these hormones are usually reduced during periods of food deprivation as a means to conserve energy (22). Fasting pups have been shown to decrease their absolute basal metabolic rates (26), apparently in an effort to conserve energy, suggesting that thyroid hormones may be reduced during this period. Also, the increase in fat oxidation during the fast may induce a decrease in plasma leptin, because leptin has been reported to reflect changes in body fat (6).

Previous studies have examined the effects of prolonged fasting in elephant seal pups on important biochemical parameters (7) as well as on insulin and glucagon (15), which are normally affected by periods of prolonged food deprivation. These studies demonstrated that fasting pups maintain biochemical homeostasis that appears to be independent of insulin and glucagon. Therefore, to provide further insight to our understanding of the physiological mechanisms underlying the adaptation to prolonged periods of natural fasting, plasma cortisol, thyroid hormones, and leptin, which are all associated with changes in fat metabolism during periods of food deprivation, were examined during the postweaning fast in NES pups. Because fat metabolism is increased during the postweaning fast, we hypothesized that plasma cortisol would increase, whereas thyroid hormones and leptin would decrease. This study should help elucidate the endocrine regulation of fasting energetics during this period in a species adapted to prolonged fasting such as the NES.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
HORMONAL CHANGES IN FASTING SEALS

METHODS

Animals and blood sampling. Fifteen NES pups (9 males, 6 females) from Ano Nuevo State Reserve (~30 km north of Santa Cruz, CA) were serially sampled in the field over the first 49 days of their postweaning fast. Only 9 of the 15 pups could be weighed at the beginning of the study, averaging 112 ± 25 kg (±SD; range 65–140 kg). Because animals were left in their natural habitat between sampling periods, not all animals could be found on each designated blood-sampling day, which resulted in a reduction of sample size over the 49-day period. For example, of the original 15 pups sampled on the first day of the study, only 7 could be found on day 49 for resampling.

Mother-pup pairs were individually identified to determine the date of weaning. A pup was considered weaned when its mother was not seen on the subsequent day. Blood samples were not collected from pups before weaning to avoid disturbing nursing mother-pup pairs. Therefore, an initial blood sample was taken within the first 24 h postweaning and used to represent preweaned, “nonfasting” conditions. Subsequent blood samples were obtained on days 7, 21, 35, and 49 of the fast. Blood sampling time was recorded and was equivalent to total restraint time, because sampling time began the moment the study animal was initially touched and ended when the blood collection tube was filled. All blood samples (8 ml) were obtained from the hind flipper, as previously described (23), and were collected into one prechilled heparinized vacutainer. After gentle rocking of the tubes in the shade, they were placed on ice in a portable ice chest until heparinized vacutainer. After gentle rocking of the tubes in the shade, they were placed on ice in a portable ice chest until they could be returned to the lab to be centrifuged, which was within 5–6 h. Blood samples were then centrifuged for 15 min (1,500 g at 4°C), and plasma was collected and frozen at −20°C for later analyses.

Hormone and other plasma analyses. In the present study, cortisol, total triiodothyronine (tT₃), total thyroxine (tT₄), free thyroxine (fT₄), and leptin were assayed with commercially available RIA kits and were validated for NES plasma. All samples were analyzed in duplicate and run in a single assay. Sera diluted pools were significantly parallel to the standard curve for each assay. Characteristics of %recovery of radioinert hormone from plasma pools and intra-assay %coefficient of variation are summarized in Table 1.

Blood urea nitrogen (BUN), creatinine, cholesterol, glucose, triglycerides, and total proteins were measured on a clinical autoanalyzer (Roche Diagnostics, Somerville, NJ). Characteristics of %recovery of radioimmunoassays

<table>
<thead>
<tr>
<th>Hormone</th>
<th>%Recovery</th>
<th>Intra-Assay %CV</th>
<th>Vendor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol</td>
<td>98.2 ± 2.3</td>
<td>&lt;5%</td>
<td>DPC, Los Angeles, CA</td>
</tr>
<tr>
<td>Leptin</td>
<td>92.3 ± 3.7</td>
<td>&lt;8%</td>
<td>Linco, St. Charles, MO</td>
</tr>
<tr>
<td>tT₃</td>
<td>95.5 ± 2.9</td>
<td>&lt;6%</td>
<td>DPC</td>
</tr>
<tr>
<td>tT₄</td>
<td>97.7 ± 4.8</td>
<td>&lt;5%</td>
<td>DPC</td>
</tr>
<tr>
<td>fT₄</td>
<td>101.5 ± 1.6</td>
<td>&lt;10%</td>
<td>DPC</td>
</tr>
</tbody>
</table>

Values are means ± SE. tT₃, total triiodothyronine; tT₄, total thyroxine; fT₄, free thyroxine.

RESULTS

Because animals were sampled in their natural habitat and moved freely about the park, recapturing pups for subsequent blood sampling on the designated days (especially after the third week postweaning) was difficult despite every attempt to find them. Therefore, sample size was reduced after the third week. Blood sampling times ranged between 2 and 16 min with a mean of 6.5 ± 0.4 min (n = 61 samples). Plasma cortisol was not positively correlated with blood-sampling time on each designated sampling day or with blood-sampling time collectively over all sampling days. Significant increases in cortisol, tT₃, and tT₄ were observed over the 49 days, with each hormone exhibiting a linear (Fig. 1), parabolic (Fig. 2), or logarithmic (Fig. 2) trend, respectively. tT₃-to-tT₄ ratio decreased linearly over the 49 days (Fig. 2). fT₄ was elevated on days 21 and 49 (Table 1), whereas the percentage of fT₄ to tT₄ remained constant throughout the fast (0.027 ± 0.001%). Means for plasma total proteins and fT₄ were significantly and negatively correlated over the 49-day period (Fig. 3). Plasma leptin at the beginning of the fast (day 1, 1.31 ± 0.24 ng/ml) was not significantly altered during the fast (day 35, 1.00 ± 0.24 ng/ml). BUN and creatinine were reduced as of day 35 and day 49, respectively (Table 2). Concentrations of plasma cholesterol and glucose were not altered over the fasting period, whereas changes in triglyceride levels were variable (Table 2).

DISCUSSION

Although some terrestrial mammals such as hibernating bears (21) or wintering deer (20) experience longer bouts of aphagia or hypophagia as a natural component of their life history, the 12-wk postweaning fast by NES pups initiated at ~30 days of age may be unrivaled among mammals. In NES pups, many of the physiological mechanisms that allow them to with-
stand such a protracted period of complete abstinence from food and water so early in life have been well described (1, 4, 23, 26). However, the hormonal changes associated with these mechanisms are not well defined. The present study reports the potential importance of cortisol to the previously reported dependence on fat metabolism that allows NES pups to fast for prolonged periods as well as the effects of prolonged fasting on thyroid hormone metabolism.

Forced fasting in mammals is usually associated with an elevation in glucocorticoids (3, 28), which induce both lipolytic and proteolytic activity to provide substrates for hepatic gluconeogenesis (10). The increase in plasma cortisol in the present study suggests that this glucocorticoid may contribute significantly to the reported increase in fat oxidation during the fast. The maintenance of relatively high glucose concentrations observed in the present study suggests that cortisol may also support carbohydrate metabolism. Pups were previously reported to be insensitive to insulin during the fast (15); therefore, glycerol liberated from cortisol-induced lipolysis may be shuttled into hepatic gluconeogenesis (10, 11). Elevated cortisol concentrations may also serve as a cue to terminate fasting and initiate feeding. Increased cortisol-induced lipolysis may result in fat mass reaching a critical lower limit at the end of the fast that may stimulate the pups to depart from the rookery and to begin foraging. Glucocorticoids have been shown to promote feeding behavior by stimulating the hypothalamus (12).

Although elevated glucocorticoids also exhibit proteolytic activity, the reductions in BUN, creatinine, and total proteins in the present study support the previously reported decrease in protein catabolism in fasting NES pups (1), despite the observed increase in cortisol. Mean triglycerides in the present study were variable over the 49 days of the fast, but they were similar to those previously reported for fasting NES pups, which were significantly lower than those for nursing NES pups (7). This reduction in triglycerides suggests that use of triglycerides by muscle is increased and storage in adipose tissue is decreased. Therefore, it is possible that proteins are protected from proteolysis during the fast by a tissue-specific increase in lipolysis that would spare lean tissue in the presence of elevated glucocorticoids.

Aside from providing energy via lipolytic pathways, the increase in cortisol observed in the present study may also serve to maintain acid-base homeostasis. The observed increase in cortisol in the present study may contribute to the synthesis of Na\(^+\)/K\(^+\)-H\(^+\)-ATPase as a potential mechanism to abolish metabolic acidosis associated with fasting, because glucocorticoids have been reported to regulate renal ATPase transcription (32). The increase in aldosterone over the course of the fast was previously suggested to contribute to an increase in renal Na\(^+\)/K\(^+\)-H\(^+\)-ATPase, because an increase in Na\(^+\) and K\(^+\) resorption from the filtrate was calculated (25) in the presence of an increase in excreted H\(^+\) (1). During conditions of acidosis, synthesis of H\(^+\)-related ATPases is increased to correct acid-base imbalances (31, 33). Fasting pups do not exhibit signs of metabolic acidosis (16), which may be attributed to an increase in mineralocorticoid (25)- and glucocorticoid-induced H\(^+\)-related ATPase synthesis.

![Fig. 2. Means ± SE plasma total triiodothyronine (tT₃) and thyroxine (tT₄) and tT₃-to-tT₄ ratio over 49 days of fasting in northern elephant seal pups. Numbers in parentheses denote the sample size (1 sample per animal) for that sampling period. Regressions were considered significant at P < 0.05.](image)

![Fig. 3. Correlation between mean ± SE plasma total proteins and free thyroxine (fT₄) in fasting northern elephant seal pups. Numbers in parentheses next to data points denote the days of fasting. Regression was considered significant at P < 0.05.](image)
The lack of a correlation between cortisol concentrations and blood-sampling time at each sampling period individually and collectively suggests that the observed increase in cortisol was induced by fasting duration and not an artifact of handling stress. Also, mean cortisol concentrations measured previously (14) and independent of our data fall remarkably close to the regression determined in the present study (Fig. 1), further suggesting that the increase in plasma cortisol is a consequence of fasting duration and not handling stress.

Starvation or reduced food intake is typically associated with reduced thyroid hormone concentrations resulting in a decrease in metabolic rate and activity level (5, 9). However, in the present study, an elevation in \( tT_3 \), \( tT_4 \), and \( fT_4 \) was observed over the course of the fast, despite the previously reported decrease in absolute metabolic rate (26). This elevation in thyroid hormones during a period of restricted food intake is not novel amongst marine mammals. West Indian manatees also exhibited an increase in thyroid hormone concentrations during an acute (19 days) period of restricted food intake (24). Therefore, the thyroid hormone responses observed in the present study and in manatees may be typical of marine mammals during periods of fasting or reduced food availability, which is a normal component of their life history.

The observed increases in both total and free thyroid hormones may not reflect increased synthesis and release, but rather alterations in clearance from circulation, in binding kinetics of the free fraction, and/or in deiodination. In hibernating ground squirrels (Spermophilus richardsoni), the increase in thyroxine (\( T_4 \)) was attributed to greatly reduced clearance rates (8), which may be likely in the present study. Also, hibernating squirrels exhibited an increase in total thyroid hormones that was attributed to an increase in binding capacity and affinity, because the percentage of free fraction was reduced (18). However, in the present study, the percentage of \( fT_4 \) to \( tT_4 \) was not changed when total protein concentrations were reduced, suggesting that the free fraction was increased due to a decrease in binding proteins (17, 29). The decrease in \( tT_3 \)-to-\( T_4 \) ratio over the 49 days is similar to that observed in food-restricted manatees (24) and suggests that deiodination of \( T_4 \) was decreased (30, 34). Reduced deiodination may serve as a mechanism to protect target tissues from the oxygen-demanding processes induced by the cellular actions of triiodothyronine (\( T_3 \)) (22). Therefore, the increase in thyroid hormones during the postweaning fast in NES pups appears to be associated collectively, or in part, with 1) a decrease in clearance from circulation, 2) a decrease in binding of the free fraction, and 3) a decrease in deiodination of \( T_4 \).

The presence of elevated thyroid hormones in circulation may permissively support fat metabolism via their lipolytic functions. The permissive effects of thyroid hormones on lipolysis have been well documented (5). Therefore, the elevated concentrations of thyroid hormones suggest that these hormones may also be involved in fat metabolism. However, the hormones can only be functional if the number of nuclear receptors they bind is not significantly reduced during the fast. Hibernating squirrels also exhibited increased thyroid hormones during hibernation, however, a reduction in nuclear receptors makes the squirrels resistant to the actions of the hormones, primarily \( T_3 \) (19).

Prolonged fasting results in a reduction in body mass and body fat, which is known to induce a decrease in circulating leptin concentrations in mammals (2, 6). During the postweaning fast in NES pups, body mass and body fat content are reduced (26), however, a significant decrease in plasma leptin was not detected in the present study. This relationship between reduced body fat and unaltered leptin concentrations suggests that in fasting pups, leptin may not be signific-

### Table 2. Plasma parameters for 15 northern elephant seal pups sampled during the first 49 days of their postweaning fast

<table>
<thead>
<tr>
<th></th>
<th>Days of Fasting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>N</td>
<td>15</td>
</tr>
<tr>
<td>( fT_4 ), pg/ml</td>
<td>5.6 (0.5)</td>
</tr>
<tr>
<td>Blood urea nitrogen, mM</td>
<td>4.0 (0.2)</td>
</tr>
<tr>
<td>Creatinine, ( \mu )M</td>
<td>97 (3)</td>
</tr>
<tr>
<td>Total protein, mg/dl</td>
<td>8.2 (0.1)</td>
</tr>
<tr>
<td>Cholesterol, mM</td>
<td>9.3 (0.3)</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>161 (11)</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>8.0 (0.2)</td>
</tr>
</tbody>
</table>

*Significantly \( P < 0.05 \) different means (SE in parentheses) from day 1 of fasting. \( N \), 1 sample per pup.

### Table 3. Summary of generalized changes in cortisol and thyroid hormones associated with starvation in humans, hibernation in squirrels or bats, and fasting in northern elephant seal pups (this study)

<table>
<thead>
<tr>
<th></th>
<th>Starvation</th>
<th>Hibernation</th>
<th>Fasting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol</td>
<td>↑ (3)</td>
<td>↑ (13)</td>
<td>↑</td>
</tr>
<tr>
<td>( tT_3 )</td>
<td>↓ (9)</td>
<td>↑ (8)</td>
<td>↑</td>
</tr>
<tr>
<td>( tT_4 )</td>
<td>↓ (9)</td>
<td>↑ (8)</td>
<td>↑</td>
</tr>
<tr>
<td>( fT_4 )</td>
<td>↓ (9)</td>
<td>↓ (18) ↑ (8)</td>
<td>↑</td>
</tr>
<tr>
<td>Clearance</td>
<td>↓ (9)</td>
<td>↓ (18)</td>
<td>↑</td>
</tr>
</tbody>
</table>

Number in parentheses indicates source. Arrows indicate general direction of concentrations or activity. Arrow with question mark indicates possible trend.
icantly involved in the regulation of body fat or in alleviating the inhibition on neuropeptide Y, which initiates the signal to begin feeding (2, 6). Leptin concentrations in the present study are the first reported for any marine mammal and are similar to those reported for fasted mice (2) and on the lower range for lean humans (6).

In summary, cortisol increased linearly over at least the first 8 wk of fasting (including cortisol data from Ref. 14), whereas total and free thyroid hormones increased significantly after the first week of fasting and remained elevated at least until the seventh week. The increase in cortisol likely contributed to the previously reported increase in fat metabolism (4, 23) and may play a significant role in carbohydrate metabolism via gluconeogenesis as indicated by the relatively high and constant glucose concentrations observed during the 7 wk of the fast. Therefore, cortisol may have replaced the pancreatic hormones in regulating carbohydrate metabolism and leptin as a potential cue to terminate fasting and initiate feeding. Elevated thyroid hormone concentrations during the fast in NES pups may be the consequence of decreased clearance and deiodination. Leptin does not appear to play a significant role in fat metabolism or in signaling the termination of the fast to initiate feeding. The present study suggests that 1) cortisol plays an integral part in the regulation of fat and, very likely, carbohydrate metabolism during the postweaning fast in NES pups and 2) thyroid hormone kinetics and metabolism are altered during the fast in a similar manner to hibernation in other mammals.

**Perspectives**

The complete abstinence from food and water may fall under one of three physiological conditions: 1) force fasting (starvation), 2) hibernation, and 3) natural fasting. Although humans and rodents may be acutely fasted, they quickly begin to exhibit pathological signs of starvation. Hibernating mammals do not generally exhibit signs of starvation, however, they remain metabolically quiescent. Seals do not exhibit the pathologies of starvation (except for prematurely weaned pups) while remaining metabolically active throughout the course of their prolonged fasting periods. These distinctions must be made when comparing the physiological responses to abstinence from food and water. However, little data exist on the endocrine changes in metabolically active, naturally fasting mammals. The focus of the present study was to observe the effects of prolonged fasting under natural conditions on hormones not previously examined to help elucidate the physiological adaptations underlying this remarkable fast. The endocrine responses observed in the present study are most similar to those for hibernating mammals (Table 3) (8, 18, 19, 21), suggesting that animals that experience extended periods of restricted dietary intake as a natural component of their life history have adapted physiologically in a similar manner. Postweaned elephant seal pups provide an interesting model to study the endocrinology in a metabolically active mammal fasting for an extended period under natural conditions.

We thank D. S. MacKenzie for reviewing this manuscript. We also thank D. Crocker, D. House, S. Kohin, D. Noren, P. Webb, and the many students of the winter field class (Biology 141) from the Univ. of California Santa Cruz (UCSC) for assistance throughout the study.

This research was supported by National Institutes of Health Minority Access to Research Careers Grant GM 58903–01 (C. L. Ortiz), National Aeronautics and Space Administration (NASA) Graduate Student Research Program NGT-2–52230 (R. M. Ortiz), and NASA Grants 121–10–50 and 121–40–10 (C. E. Wade).

This research was conducted in partial fulfillment of the requirements for the doctorate degree in biology for R. M. Ortiz. Research was conducted under National Marine Fisheries Service marine mammal permit #836 to C. L. Ortiz and UCSC Chancellor's Animal Research Committee permit Orti89.08 to C. L. Ortiz and R. M. Ortiz.

**REFERENCES**

16. Kohin S. Respiratory Physiology of Northern Elephant Seal Pups: Adaptations for Hypoxia, Hypercapnia and Hypometabo-


