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Protein catabolism in suckling and fasting northern elephant seal pups (*Mirounga angustirostris*)

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**Abstract** Nursing elephant seal pups are hypothesized to be preadapted to the postweaning fast, yet no comparison of lipid or protein use for meeting metabolic costs has been made between these contrasting nutritional periods. To address this, protein catabolism was estimated in five elephant seal pups from measurements of urea turnover made twice during nursing and twice during the postweaning fast. Changes in body composition were measured in ten separate weaned pups via tritiated water dilution and matched to fasting urea turnover measurements in order to assess errors in protein catabolism derived from urea turnover rates. Estimates of lean mass loss based upon urea turnover and tritiated water dilution were in general agreement, supporting estimates of protein catabolism derived from urea turnover measurements. Protein catabolism was estimated to contribute less than 4% to the average metabolic rate of suckling and fasting pups implying strict protein conservation during both periods and supporting the hypothesis that suckling pups are preadapted to fasting. It is proposed that strict protein conservation across suckling and fasting compensates for relative reductions in maternal investment associated with the abbreviated lactation period of the elephant seal.

**Keywords** Development · Fasting · Maternal investment · Protein catabolism · Urea turnover

**Abbreviations** AMR average metabolic rate · B:C ratio of BUN to creatinine · BUN blood urea nitrogen · HTO tritiated water · MWUN milk water urea nitrogen · PA plasma activity · $N_\text{p}$ urea pool size · $R_\text{a}$ average daily turnover of urea · $TBW$ total body water

**Introduction**

Northern elephant seals incorporate fasting as an integral component of terrestrial activities such as breeding, molting, lactation, and as pups, postweaning development. These activities are energetically demanding and, though other factors may contribute to the termination of fasting while nutrient reserves exist to support its continuation, e.g., no further breeding opportunities for males, fasting duration is ultimately limited by the amount of body stores available to meet metabolic costs. Elephant seals may fast for up to 3 months while engaging in reproductive behavior or postweaning development, and as a result, they have been the subjects of numerous investigations on metabolic adaptations to fasting. Elephant seal pups in particular have been of interest to researchers because of their relative ease of handling during the postweaning fast. Pups are suckled for approximately 25 days before being abruptly weaned and, incapable of effectively diving and swimming, fast on land for 2–3 months. During this time they continue neonatal development and acquire the motor skills necessary for making their first trip to sea (Reiter et al. 1978). This period of substantial tissue reorganization without exogenous nutritional input makes the weaned elephant seal pup a model system for studying physiological adaptations to fasting under the constraints of development.

Elephant seal pups progressively reduce their metabolic rate throughout the postweaning fast losing an average of 0.9 kg mass/day during the first 2 weeks of fasting and approximately 0.5 kg/day for the remainder of the fast (Rea and Costa 1992). Isotope turnover studies and changes in urine composition with time...
fasting suggest that elephant seal pups meet less than 4% and 1% of their average metabolic rate (AMR) through protein (Adams and Costa 1993; Castellini et al. 1987; Pernia et al. 1980) and glucose oxidation (Keith and Ortiz 1989), respectively. Results from free fatty acid turnover experiments, water flux measurements, and oxygen consumption measurements imply that metabolic requirements are primarily met through fatty acid oxidation (Castellini et al. 1987; Ortiz et al. 1978; Rea and Costa 1992) leaving protein stores available for the de novo synthesis of lean tissue. This manner of partitioning body stores to meet metabolic costs is common to fasting mammals and has been termed Stage II fasting.

Kirby and Ortiz (1994) suggested that suckling elephant seal pups are preadapted to the postweaning fast and exhibit metabolic characteristics during suckling that are similar to those observed during fasting. This proposition was based upon the observation that suckling pups consume large quantities of maternal milk with negligible carbohydrate and high lipid content and rapidly assimilate fat into body mass. Kirby and Ortiz argued that consumption of large quantities of lipid suppresses insulin secretion, thereby diminishing the ability to utilize glucose, and that because carbohydrate is a negligible constituent of maternal milk the ability to use consumed carbohydrate as a fuel source is pre-empted. These factors should collectively contribute to a similar partitioning of body stores (e.g., fat vs. protein) to meet metabolic costs between feeding and fasting. Glucose clearance and insulin tolerance tests performed on suckling and fasting pups suggest that lipid release from adipose tissue is maximized and glucose oxidation is minimized during both periods, indirectly supporting the fasting preadaptation hypothesis (Kirby and Ortiz 1994). However, no comparison of the contribution of lipid or protein to the metabolic rate between these periods has been made to address whether different body stores are similarly partitioned to meet metabolic costs.

The purpose of this study was to compare the use of body stores to meet metabolic costs in suckling and fasting elephant seal pups. To this end, protein catabolism was estimated from parameters derived in the noncompartmental analysis of urea kinetics (Crocker et al. 1998; Pernia et al. 1980; Wolfe 1992). In order to address errors in protein catabolism estimates, body composition measurements were made and matched in time to measurements of urea kinetics during fasting. Calculated loss of lean mass using each method was compared. Protein catabolism estimates for both suckling and fasting periods were then used to address the hypothesis that suckling elephant seals are preadapted to the postweaning fast.

Materials and methods

Urea turnover in suckling pups and weanlings

Urea turnover studies in suckling and fasting northern elephant seal pups were conducted at Año Nuevo State Reserve, San Mateo County, California, during the 1995–1996 breeding season. Five pups born to known-age adult females were chosen within 2 days of parturition and marked with bleach for future identification. Pups were re-marked with hair dye (Lady Clair; Stamford, Conn.) after molting in order to maintain identification throughout the postweaning fast. Because weaning seals move more freely about the rookery late in the fast, radio transmitters (148–150 MHz; Advanced Telemetry Systems, Isanti, Minn.) were attached to the pelage of seals during the 8th week of the fast to facilitate daily relocation. All pups exhibited behavior typical of healthy elephant seal pups and departed to sea at the end of the fast.

Using a bolus injection of [14C]-urea a noncompartmental model was used to describe urea kinetics in each pup (Wolfe 1992). This technique was chosen for several reasons: (1) it allowed urea kinetics to be measured in the pup’s natural environment, (2) it reduced the separation time of suckling-age pups from mothers, which in turn reduced the possibility of pup abandonment and (3) it was previously used to study urea kinetics in both fasting elephant seal adults and pups, thus reducing the error in inter-study comparisons resulting from different techniques.

Urea turnover was measured in each pup a total of four times—twice during suckling and twice during the postweaning fast. Since food composition is known to affect the partitioning of metabolic substrates to meet energy needs (Robbins 1993), sampling periods during suckling were selected according to changes in the composition of elephant seal milk (Crocker 1995; Riedman and Ortiz 1979). The first urea turnover measurement was performed at 5 days postpartum when milk lipid content was low relative to water. The second measurement was made 20 days postpartum when milk lipid exceeded 50% of maternal milk composition by mass. Fasting measurements were made during the 2nd week and 8th week of the postweaning fast in order to maximize the time interval between measurements without losing subject animals to migration.

Suckling pups were separated from their mothers on the day of [14C]-urea administration and manually restrained for injection while weaning seals were immobilized with an IM injection of tiletamine/zolazepam at a dose of 1.0 mg/kg (Telazol; Fort Dodge Labs, Ft. Dodge, Ia.). Prior to tracer injection, seals were weighed in a nylon bag attached to a 500±0.2-kg capacity load cell (Dyna-Link model; Measurement Systems International, Seattle, Wash.) and a blood sample was drawn in order to determine isotopic background activity. At 5 days postpartum, pups were administered a bolus injection of 0.1 mcI [14C]-urea in sterile injectable saline while at 20 days postpartum and during the 2nd and 8th week of the fast, pups were administered 0.3 mcI [14C]-urea. The initial blood collection and subsequent isotope administration were made via the dorsal extradural vein. The absolute quantity of tracer injected was determined by gravimetric calibration of the syringes used for isotope administration.

Repeat postinjection blood samples taken at 15-min intervals indicated that equilibration of isotope occurred within 90 min, however, pups were allowed to equilibrate for 3-h before a blood sample was collected for use in the kinetic model (Ortiz et al. 1978). Blood samples were taken daily for 2 days postequilibration during the early suckling period, 3 days postequilibration during the late suckling period, and up to 7 days postequilibration during the fasting periods. All seals were manually restrained for daily postequilibration blood samples, either by hand or with the help of a nylon restraint bag (Pernia et al. 1980), and samples were drawn from a dorsal or ventral ankle vessel. Blood samples were collected in 7-ml Na-heparin tubes, gently mixed, and placed on ice. Samples were centrifuged and the plasma obtained within 2 h of sample collection.

Aliquots of plasma (100–800 µl) were placed in 5-ml of Ecolite scintillation cocktail (ICN; Costa Mesa, Calif.) and the plasma activity determined using standard liquid scintillation techniques on a Beckman LS 6500 multi-purpose liquid scintillation counter. A counter-determined quench correction factor was established for each sample by determining the activity of a series of [14C] standards with identical activity but with variable degrees of quench.