Age, Sex, and Reproductive State Influence Free Amino Acid Concentrations in the Fasting Elephant Seal

Dorian S. Houser
Daniel E. Crocker
Department of Biology, School of Science and Technology, Sonoma State University, 1801 East Cotati Avenue, Rohnert Park, California 94928

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ABSTRACT

Long-term fasting is a component of northern elephant seal (*Mirounga angustirostris*) life history requiring physiological adaptations to nitrogen conservation. Plasma free amino acids (FAAs) were determined for five elephant seal pups during the second and eighth weeks of the postweaning fast, six lactating female seals at 4–6 and 25 d postpartum, and seven sexually competitive adult male seals taken midway through the breeding season. Total FAAs declined in lactating females (11%) and pups (30%) with time fasting, but cystine concentration more than doubled in pups while decreasing by ~43% in lactating females. Methionine concentration significantly increased (~68%) across lactation in adult females but was low for all classes of seal. Alanine was the most abundant FAA in adult males, and glycine became the dominant FAA in adult females late in lactation. Glutamine dominated the FAAs of weaned pups across the fast. Reductions in total FAAs of weanlings mirrored reductions in protein catabolism, but reductions in total FAAs also occurred in lactating females concomitant with an increase in protein catabolism. Observed variation in FAA concentrations may reflect ontogenetic requirements for certain amino acids in fasting weanlings. Similarly, increases in specific FAA concentrations across lactation may reflect variations in FAA flux resulting from the nutrient demands of lactogenesis.

Introduction

Northern elephant seals (*Mirounga angustirostris*), like many phocid seals, combine fasting with terrestrial residency during breeding and molting periods. Elephant seals mobilize endogenous nutrients for the production of new pelage during the molt (Slip et al. 1992; Worthy et al. 1992), and seals of reproductive age engage in energetically costly activities during the breeding season, such as combative male-male competition or lactogenesis (Le Boeuf 1974; Costa et al. 1986; Kretzmann et al. 1993; Crocker 1995). Elephant seal pups fast after weaning and can spend 2–3 mo de novo synthesizing lean tissue through the mobilization of existing protein stores and developing the diving skills necessary for survival at sea (Thorson and Le Boeuf 1994). Lactogenesis, pelage synthesis, postnatal development, and increased energy expenditure associated with combative mating practices can each increase demands on the utilization of limited nutrient reserves. Given that each of these activities will have different nutrient demands, various metabolic controls must exist to balance the nutrient requirements of these activities against the maintenance energy requirement of the animal.

The mass of adult elephant seals arriving for breeding and molting and pups at the beginning of the postweaning fast consists of ~35%–50% fat (Rea and Costa 1992; Crocker et al. 1998; Houser and Costa 2001; D. E. Crocker and D. S. Houser, unpublished data). Large fat stores facilitate reductions in the rate of protein loss, but do not prevent it (Goodman et al. 1980; Cherel et al. 1992, 1993; Atkinson et al. 1996; Cherel and Groscolas 1999). In fasting weanlings, protein catabolism is estimated to account for less than 4% (Pernia et al. 1980; Adams and Costa 1993) of the daily metabolic expenditure and may be as low as 1% late in the fast (Houser and Costa 2001). In contrast, lactating females lose the ability to spare protein as the fast progresses. The proportion of the daily metabolic rate that is due to protein catabolism increases from 8% to 11% from mid- to late lactation (Crocker et al. 1998). Little is known of how protein catabolism varies with time fasting in reproductively competitive males. However, to gain mating access to females, males may engage in physical combat for periods of up to 3 mo (Le Boeuf 1974). Increased glucose oxidation may be required to meet the energetic cost associated with combat, placing a greater demand on protein reserves to supply gluconeogenic precursors. Regardless of age or sex, fasting precludes replacement of catabolized protein through feeding. Since protein consists of many nitrogen-containing amino acids (AAs), protein loss associated with fasting results in a negative nitrogen (N) balance through AA catabolism and subsequent excretion of nitrogenous wastes (e.g., urea). Further commitment of protein stores to metabolically demanding activities (e.g., lactation, development) should magnify the loss of N

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above that lost to maintenance metabolism and increase the economy of N stores.

The management of N should be dictated by metabolic demands particular to age, sex, and reproductive state. Postpartum females must meet both their own metabolic requirements and, through milk production, the nutrient requirements of their pups (Kovacs and Lavigne 1986; Boness and Bowen 1996). Lactogenesis places a large demand on both lipid and protein reserves, as produced milk is copious and energy-rich, with negligible amounts of carbohydrate (Riedman and Ortiz 1979). The physiological maturation of weanlings imposes restrictions on protein loss so that lean tissue may be synthesized de novo (Thorson and Le Boeuf 1994). Adult males face neither the accessory demands of offspring nor rapid developmental requirements, but the high level of activity associated with breeding is both prolonged and strenuous, potentially imposing greater glucose requirements and accelerating the loss of AAs to gluconeogenesis. Given the differential demands of these age- and sex-specific activities, varying from primarily energetic requirements to those having structural requirements (i.e., transfer of AAs to pups or utilization in tissue structuring), management of N balance and specific AAs should also differ by age, sex, and reproductive state.

The purpose of this article is to investigate possible differences in the management of N balance by assessing the concentration of AAs within the plasma of elephant seals. It provides the first measurement of a large number of plasma AAs in breeding adult males, lactating females, and developing weanlings. Furthermore, comparisons are made between measurements taken early and late in lactation (i.e., first versus last week) and early (second week) and late (eighth week) in the postweaning fast, thus providing insight into the management of N balance as nutrient reserves are increasingly depleted.

Material and Methods

All samples were collected from seals at the Año Nuevo State Reserve, San Mateo County, California. Collections from adult males and lactating females were obtained during the 1998 breeding season. Collections from weanlings occurred between the late winter and early spring of 1997, during the postweaning fast. Soon after arriving on land adult males were marked with hair bleach, while adult females were marked with blue-black hair dye (Procter and Gamble–Clairol, Stamford, Conn.). Parturition dates were noted for all marked females, and females and pups were monitored daily throughout lactation for normal mother-pup interactions. Pups from which samples were collected in 1997 were identified as subjects at the time of weaning and marked with hair dye following the postweaning molt.

Seals were chemically immobilized through the intramuscular administration of tiletamine/zolazepam (Telazol; Fort Dodge Labs, Fort Dodge, Iowa). Samples collected from adult females were made during a study on the foraging ecology of elephant seals (Le Boeuf et al. 2000), while samples collected from fasting pups were made during a study on body composition changes across the fast (Houser and Costa 2001). The reader is referred to these articles for specifics on chemical immobilization. Adult males were administered an intramuscular injection of tiletamine/zolazepam at an initial dose of 0.3–0.4 mg/kg and titrated upward until immobilization was attained. Blood collections from adult females were made 4–6 d postpartum (early lactation) and 25 d postpartum (late lactation). A total of six adult females were sampled, with repeat samples taken on four of the individuals; an additional individual was sampled during early lactation and another during late lactation. Collections from fasting weanlings were made during the second (early fasting) and eighth (late fasting) weeks of the postweaning fast. A total of five weanlings were sampled, with repeat samples taken on all individuals. The exact time spent at the rookery at the time of blood sampling could not be determined for adult males, but all samples were taken near the middle of January during the 1998 breeding season. A total of seven adult males were sampled during this time. All procedures conducted in this study were approved by the Institutional Use and Care of Animals Committee of the University of California, Santa Cruz, and comply with the "Principles of Animal Care," publication 86-23 of the National Institutes of Health.

Blood samples were collected into 7-mL Na-heparin plasma tubes via an 18-g spinal needle inserted between the vertebrae and into the extradural vein. Following centrifugation, aliquots of plasma were drawn off and deproteinated with a 1 : 5 ratio of 10% 5-sulfylsalycylic acid solution to plasma (Lee and Slocum 1988). Samples were vigorously agitated and placed on ice. Within 1 h of treatment plasma samples were microcentrifuged at 10,000 rpm and the supernatant withdrawn. Deproteinated plasma samples were stored at −20°C until they were analyzed for free amino acid (FAA) fractions.

Chromatography

Identification and concentration of all plasma FAAs were determined at the Molecular Structure Facility (MSF) of the University of California, Davis, according to the procedures of Lee and Slocum (1988). The MSF used a Beckman 6300 AA analyzer with a lithium citrate buffer system, and all analyses and reports were made with Beckman 32 Karat software. Briefly, the system employs ion-exchange chromatography for the separation of AAs and then applies a ninhydrin reaction to produce a colored complex that is spectrophotometrically analyzed. A known quantity of α-guanidino-propionic acid (AGPA; 200 nmol/mL) is added as an internal standard to the buffer solution so that variation in the volume of the injected sample can be determined. Using a standard of 5 nmol of AA on column, the analyzer can be calibrated to within a 7% error. The limit of detection is 50–200 pmol, depending on the in-

dividual AA assayed, and the error increases as AA concentrations decline below 500 pmol.

For all plasma samples, 100 λ of deproteinated plasma was combined with 100 λ of AGPA diluted buffer, and 50 λ of the new solution was injected into the AA analyzer. Plasma concentrations were determined for the following AAs: aspartic acid (asp), hydroxyproline (hypro), threonine (thr), serine (ser), asparagine (asn), glutamic acid (glu), glutamine (gln), cystine (cys), methionine (met), proline (pro), glycine (gly), alanine (ala), valine (val), isoleucine (ile), leucine (leu), tyrosine (tyr), phenylalanine (phe), ornithine (orn), lysine (lys), histidine (his), and arginine (arg). Tryptophan is usually present at low levels. However, it has the lowest response factor of all the common AAs, and there was typically insufficient tryptophan present for its detection.

Statistical Analysis

The accuracy of a peak ID, or the error involved in the calculation of a specific AA quantity using the Beckman 6300 system, was occasionally questionable. This occurred because there were insufficient quantities of the AA or overlapping peaks from coeluting contaminants. Whenever values for a particular AA had a greater than 10% error, because of low quantity or interference from coeluting contaminants, the value for the AA was excluded from analysis. (Note the unbalanced n for some AAs listed in Tables 1–3.) If each FAA analysis is considered separately, elimination as a function of insufficient quantity or the presence of coeluting contaminants occurred ~5% of the time. Errors were biased to particular species of AA; 56% of all tyrosine measurements and 37% of all methionine measurements were not reportable. Errors in the measurement of these FAAs constituted the greatest fraction of measurement errors.

Weanlings. Differences between AA concentrations measured during the second and eighth weeks of the postweaning fast were determined by a t-test for dependent measures ($\alpha = 0.05$). Where the sample size (n) was unbalanced because of processing errors, only matched samples were used in the comparison. FAA concentrations measured during the second and eighth weeks of the postweaning fast were also compared to concentrations measured in adult females during early and late lactation, respectively, using a t-test and assuming equal variances.

Adult Females. Not all samples taken between the early and late lactation periods were obtained from the same female; that is, the samples were not completely matched. Therefore, differences between FAA concentrations measured during these periods were determined by a t-test for dependent samples ($\alpha = 0.05$) using only the females for which matched samples were obtained ($n = 4$).

Adult Males. Samples from adult males were taken at a single point during the middle of the breeding season (January 1998). The exact amount of time each male spent on the rookery was unknown, as not all males were marked the day they arrived at the rookery. However, residency was assumed to be comparable between males based on the observed time at the rookery and similarities in body size and mass. Plasma AA concentrations from all males were pooled to determine means and standard deviations for each AA. Males were not statistically compared to adult females or weanlings because of differences in the sampling schemes (i.e., the middle of the breeding season, as opposed to early or late in lactation or the postweaning fast).

Results

Weanlings

Concentrations of plasma FAAs in weanling seals measured during the second and eighth weeks of the postweaning fast are presented in Table 1. Several AAs demonstrated no change in plasma concentration across the fast. Of those that significantly changed, cystine increased with duration fasting, rising from 10.9 to 23.6 nmol/mL over the 6-wk period, an increase of 116%. Reduction was ≥40% for several of the AAs (aspartic acid, asparagine, glutamic acid, alanine, valine, and histidine) but was more modest for glutamine (16%) and proline (19%). Aspartic acid and glutamic acid concentrations measured early in the postweaning fast were significantly greater than in fasting adult females early in lactation (aspartic acid: $t = 5.9$, $P < 0.01$; glutamic acid: $t = 4.8$, $P < 0.01$), but concentrations late in the postweaning fast were similar to those of adult females late in lactation. Hydroxyproline was significantly greater in weanlings than in females during both fasting measurements: early in the postweaning fast versus early lactation ($t = 4.6$, $P < 0.01$) and late in the postweaning fast versus late lactation ($t = 2.9$, $P = 0.02$). Glutamine composed the greatest fraction of the total FAAs at both points during the postweaning fast.

Lactating Females

Methionine, glycine, and tyrosine increased with the progression of lactation and fasting in adult females; methionine increased by 68% and glycine by 19% (Table 2). By day 25 of lactation the greatest portion of the plasma FAAs consisted of glycine, which exceeded glutamine as the dominant FAA and comprised nearly 19% of the total FAA concentration. Glutamine and cystine significantly declined across lactation, with the greatest reduction observed in cystine (~43%). No other significant variations in FAA concentration were observed with time lactating.

Table 1: Plasma AA concentrations for weanling elephant seals early and late in the postweaning fast

	2 wk Postweaning		8 wk Postweaning		
	nmol/mL ± SD	*n*	nmol/mL ± SD	*n*	*t*-test
Essential AAs:					
Threonine (thr)	69.21 ± 13.98	5	52.03 ± 8.84	5	NS
Valine (val)	170.69 ± 33.27	5	100.12 ± 13.16	5	$t = 5.12$, $P < .01$
Methionine (met)	6.13 ± .64	5	2.04	1	...[a]
Isoleucine (ile)	57.12 ± 16.79	5	57.67 ± 21.01	5	NS
Leucine (leu)	107.42 ± 21.44	5	74.19 ± 12.23	5	$t = 3.11$, $P = .04$
Phenylalanine (phe)	50.22 ± 4.18	5	42.82 ± 11.76	5	NS
Lysine (lys)	152.95 ± 6.74	5	108.44 ± 18.41	5	$t = 5.35$, $P < .01$
Histidine (his)	127.45 ± 18.44	5	76.87 ± 13.89	5	$t = 3.88$, $P = .02$
Arginine (arg)	109.93 ± 11.67	5	82.83 ± 16.75	5	$t = 3.72$, $P = .02$
Total essential AAs	851.12		597.01		
Nonessential AAs:					
Aspartic acid (asp)	16.16 ± 2.70	5	8.31 ± .50	5	$t = 6.76$, $P < .01$
Hydroxyproline (hypro)	38.78 ± 8.90	5	30.28 ± 8.79	5	NS
Serine (ser)	114.68 ± 12.79	5	86.76 ± 19.65	5	NS
Asparagine (asn)	37.89 ± 6.08	5	22.27 ± .86	5	$t = 5.56$, $P < .01$
Glutamic Acid (glu)	54.97 ± 16.94	5	31.59 ± 5.79	5	$t = 2.93$, $P = .04$
Glutamine (gln)	413.46 ± 62.23	5	349.31 ± 42.55	4	$t = 5.01$, $P = .02$
Proline (pro)	92.60 ± 6.64	5	74.72 ± 3.94	5	$t = 5.93$, $P < .01$
Glycine (gly)	334.50 ± 32.07	5	228.77 ± 38.75	5	$t = 4.86$, $P < .01$
Alanine (ala)	318.39 ± 46.55	5	149.31 ± 20.12	5	$t = 7.76$, $P < .01$
Cystine (cys)	10.91 ± 5.42	5	23.59 ± 6.06	5	$t = -3.60$, $P = .02$
Tyrosine (tyr)			22.82 ± 3.87	2	...[a]
Ornithine (orn)	54.94 ± 10.87	5	45.46 ± 6.77	5	NS
Total nonessential AAs	1,487.28		1,073.19		

Note. Results of *t*-tests are provided when concentrations between periods are significantly different; NS is given when differences are nonsignificant. Total AA values are calculated as the sum of the mean values for each AA.

[a] Test not conducted because of a lack of samples with measurable AA concentration.

Adult Males

AA concentrations for adult males are presented as means and standard deviations in Table 3. Alanine and glutamine collectively comprised approximately 42% of the total FAA concentration in combative adult males.

Discussion

Plasma AA profiles taken from fasting mammals provide a "snapshot" of FAA dynamics at a specific point in time during the fast. They do not indicate the fate of the AAs or their kinetics, both of which are necessary for a comprehensive understanding of an animal's N economy. Any relationships between an FAA and its putative role as a metabolite must therefore be handled with caution, as they are necessarily speculative. Through comparisons between individuals of a species in different metabolic states and between different species in a similar metabolic state, FAA profiles can provide insight that narrows the scope of potential questions to those most relevant to an animal's N economy.

Elephant seal weanlings depress protein catabolism and metabolic rate with increasing time of fasting (Rea and Costa 1992; Adams and Costa 1993; Houser and Costa 2001), but lactating females demonstrate a progressive increase in the rate of protein catabolism across lactation (Crocker et al. 1998). The mean total concentration of FAAs declined in both weanlings and lactating females as fasting progressed. Weanlings declined from 2,338 to 1,647 nmol/mL, and lactating females declined from 3,049 to 2,728 nmol/mL, a decline of 30% and 11%, respectively. In fasting weanlings, there was depression of 12 of the plasma FAAs across the fast (Table 1), five of which were essential AAs. Patterns were more variable in lactating females (Table 2), with glutamine and cystine declining and methionine and glycine increasing with time fasting.

Table 2: Plasma AA concentrations for lactating elephant seal females early and late in lactation

	Early Lactation		Late Lactation		
	nmol/mL ± SD	*n*	nmol/mL ± SD	*n*	*t*-test
Essential AAs:					
Threonine (thr)	91.07 ± 17.24	4	81.21 ± 14.49	4	NS
Valine (val)	244.68 ± 35.29	5	161.94 ± 19.60	4	NS
Methionine (met)	4.56 ± .89	5	7.64 ± 1.54	5	$t = -4.42$, $P = .02$
Isoleucine (ile)	62.53 ± 8.13	5	55.09 ± 9.40	5	NS
Leucine (leu)	139.32 ± 32.07	5	116.29 ± 23.00	5	NS
Phenylalanine (phe)	80.79 ± 3.37	5	98.85 ± 14.42	5	NS
Lysine (lys)	206.47 ± 37.27	5	213.76 ± 40.80	5	NS
Histidine (his)	90.13 ± 7.22	5	101.77 ± 14.79	5	NS
Arginine (arg)	106.88 ± 21.24	5	100.34 ± 12.92	5	NS
Total essential AAs	1,026.43		936.89		
Nonessential AAs:					
Aspartic acid (asp)	7.81 ± 1.65	5	8.03 ± 1.24	3	NS
Hydroxyproline (hypro)	20.31 ± 1.60	5	16.90 ± 2.96	4	NS
Serine (ser)	165.18 ± 45.73	5	155.47 ± 26.52	5	NS
Asparagine (asn)	40.33 ± 6.67	5	33.94 ± 5.38	4	NS
Glutamic Acid (glu)	17.91 ± 2.34	5	34.07 ± 43.48	5	NS
Glutamine (gln)	635.78 ± 108.26	5	420.88 ± 96.19	5	$t = 5.91$, $P < .01$
Proline (pro)	110.80 ± 15.28	5	89.12 ± 11.71	5	NS
Glycine (gly)	433.26 ± 88.23	5	516.56 ± 66.98	5	$t = -4.02$, $P = .03$
Alanine (ala)	442.45 ± 67.40	5	367.64 ± 35.91	5	NS
Cystine (cys)	41.46 ± 8.22	5	23.50 ± 10.54	5	$t = 3.27$, $P = .047$
Tyrosine (tyr)	41.45 ± 12.84	5	36.44 ± 5.96	4	$t = -5.27$, $P = .03$[a]
Ornithine (orn)	60.81 ± 18.46	5	88.12 ± 15.00	5	NS
Total nonessential AAs	2,017.54		1,790.67		

Note. Results of *t*-tests are provided when concentrations between periods were significantly different; NS is given when differences were nonsignificant. Total AA values are calculated as the sum of the mean values for each AA.

[a] Test run with only matching samples showed an increase in tyrosine concentration. The inclusion of an unmatched early-lactation sample (62.96 nmol/mL) results in higher values for early than for late lactation in the table. The mean value for matched samples during early lactation was 34.19 nmol/mL.

Weanlings

Elephant seal pups obtain all of the nutrients necessary to endure the postweaning fast from nursing, including sufficient quantities of the essential AAs to permit continued physiological development. Reductions in the total plasma FAA pool are consistent with observations of reduced protein catabolism that occur with time fasting (Pernia et al. 1980; Adams and Costa 1993; Houser and Costa 2001). The fate of any particular AA during postweaning development is unknown, but investigations into their rates of utilization and endpoints (i.e., catabolized or synthesized into protein) will provide insight on how development occurs in light of long-term food deprivation. Considered below are several AAs that demonstrated significant variations across the fast. Putative roles of each are given, with the hope of inspiring future research into nutrient utilization under the constraints of postnatal ontogeny and fasting.

Histidine concentration is greater in elephant seal milk than it is in multiple other mammalian species (Davies et al. 1983; Davis et al. 1995) and demonstrates a significant reduction in circulating levels across the postweaning fast. This pattern is consistent with expectations of histidine commitment to development and maintenance metabolism, given preweaning histidine accumulation. One of the most significant developmental changes occurring during the postweaning fast is a dramatic increase in blood hemoglobin and muscle myoglobin stores (Thorson and Le Boeuf 1994). Several of the AAs that declined across the fast in weanling seals provide residues that account for much of the composition of α-hemoglobin and β-hemoglobin chains (Lehninger 1993), with histidine playing a particularly important conformational role. The observed decrease in these FAA pools may reflect increased commitment to the synthesis of these respiratory pigments.

Table 3: Plasma AA concentrations for sexually competitive adult male elephant seals approximately midway through the breeding season

	nmol/mL ± SD	*n*
Essential AAs:		
Threonine (thr)	144.81 ± 19.12	7
Valine (val)	371.57 ± 71.52	7
Methionine (met)	3.72	1
Isoleucine (ile)	183.69 ± 25.18	7
Leucine (leu)	331.78 ± 66.18	7
Phenylalanine (phe)	92.46 ± 10.07	7
Lysine (lys)	252.85 ± 40.68	7
Histidine (his)	92.43 ± 13.35	7
Arginine (arg)	111.19 ± 11.96	7
Total essential AAs	1,584.51	
Nonessential AAs:		
Aspartic acid (asp)	7.03 ± 1.50	7
Hydroxyproline (hypro)	16.07 ± 5.09	7
Serine (ser)	139.27 ± 19.04	7
Asparagine (asn)	36.70 ± 2.99	7
Glutamic Acid (glu)	15.24 ± 5.01	7
Glutamine (gln)	514.26 ± 56.27	7
Proline (pro)	119.14 ± 16.74	7
Glycine (gly)	245.29 ± 41.68	7
Alanine (ala)	589.43 ± 107.20	7
Cystine (cys)	32.27 ± 4.99	7
Tyrosine (tyr)	71.72 ± 5.88	2
Ornithine (orn)	108.02 ± 29.78	7
Total nonessential AAs	1,894.45	

Note. Total AA values are calculated as the sum of the mean values for each AA.

Cystine is created from two covalently bonded cysteine molecules and is the transport form of the AA found within the plasma pool. Cysteine is typically considered a nonessential AA in adult mammals but an essential AA in neonates because of the inability of neonates to de novo synthesize it in sufficient quantities to meet maintenance metabolism and developmental demands. Milk cystine concentration falls within the range of values observed in multiple terrestrial mammal species (Davis et al. 1995), but plasma levels of cystine significantly increase across the postweaning fast. This pattern contrasts with the decline in concentration across that fast that was observed in most of the other FAAs. Cysteine is important to the development of skin, collagen, and hair (Toda et al. 1976), contributes to the structure of various globulins, and plays an important role in enzyme activation/inhibition through the provision of sulfhydryl reaction sites (Lehninger 1993). Elevated concentrations of cystine may therefore reflect a higher requirement for cysteine and altered kinetics during development. Alternative explanations for increased cystine concentration include increased protein catabolism and dehydration. Increased protein catabolism is an unlikely explanation, given that elephant seal weanlings improve protein sparing throughout the fast (Pernia et al 1980; Adams and Costa 1993; Houser and Costa 2001). Plasma water homeostasis and preservation of hematological values with time fasting indicate that elephant seal pups do not become dehydrated with time fasting (Costa and Ortiz 1982; Castellini et al. 1990), suggesting that an increase in any AA concentration due to dehydration is also unlikely.

Alanine decreased by more than 50% and demonstrated the most substantial decline of any of the FAAs measured across the postweaning fast. Being the primary AA in the gluconeogenic pathway of most mammals, the fate of alanine could provide insight on glucose management within elephant seal weanlings. Work conducted by Keith (1984) suggests that alanine contributes little to gluconeogenesis, yet fasting weanlings maintain plasma glucose at values in the range of 120–175 mg/dL (Kirby and Ortiz 1994). However, glucose flux is low (Keith and Ortiz 1989), and it has been suggested that most glucose requirements could be met through the recruitment of glycerol as a precursor to gluconeogenesis (Castellini et al. 1987). Glycerol, an end-product of fatty acid catabolism, is produced in large quantities by the elephant seal because it relies almost exclusively on fat to meet its energy needs (Ortiz et al. 1978; Castellini et al. 1987). Reliance on glycerol for gluconeogenesis may be one mechanism by which AAs such as alanine are preserved from loss to glucose production. Determining whether reduced alanine concentrations are due to increased utilization or decreased production would provide insight into whether glycerol utilization progressively protects nitrogen stores from catabolic pathways during fasting.

Lactating Females

Lactating and fasting female elephant seals are faced with the dual challenge of meeting their own metabolic demands while supplying nutrients to their pups for neonatal and postweaning development. Some variation in AA dynamics is expected, given that protein catabolism increases and both body composition and milk composition change across lactation (Riedman and Ortiz 1979; Crocker et al. 1998). Five of the twenty-one measured FAAs significantly varied between the first and fourth weeks of lactation, and of these, methionine, tyrosine, and glycine significantly increased with time lactating. Elevations in FAAs may be due to hemoconcentration; although weanling elephant seals are superb at maintaining osmotic balance during the postweaning fast and do not demonstrate a progressive dehydration with time fasting (Costa and Ortiz 1982), they are not constrained by water lost to milk production, as are lactating females. However, if hemoconcentration were occurring, it would likely be reflected in more than three of the twenty-one AAs measured. Alternative reasons for increased FAA con-

centration include increased skeletal muscle degradation, elevated rates of synthesis, and reduced rates of catabolism. Increased rates of muscle degradation are supported through estimates of protein catabolism across lactation (Crocker et al. 1998). Increased protein catabolism may result from a reduction in the availability of fat stores to meet maintenance metabolism needs, since there is an increased commitment of fat stores to milk production with time lactating (Riedman and Ortiz 1979).

Certain FAA concentrations are substantially lower than observed in the fasting black bear (*Ursus americanus*), a mammal that shares a common phylogenetic ancestry with the elephant seal (Riedman 1991) and that also combines lactation with fasting while overwintering with cubs. Denning female black bears (lactating and nonlactating) have plasma hydroxyproline concentrations ranging from ~278 to 312 nmol/mL, exceeding values measured in elephant seals by up to 19 times (Wright et al. 1999). Methionine was also much lower in elephant seals, 2.0–7.6 nmol/mL, compared to that measured in denning female black bears, ~49–78 nmol/mL (Wright et al. 1999). Variations in the flux and concentration of AAs may reflect disparities in nutrient requirements or energetic expenditures (i.e., elephant seals remain active during lactation), but given that food sources consumed by black bears and elephant seals are different in nutritional content, variation in FAAs may be indicative of food-specific nutrient accumulation before fasting.

Homocysteine can be salvaged to resynthesize methionine through a homocysteine methyltransferase catalyzed reaction, or it can be further catabolized to succinyl-CoA, the latter also resulting in the synthesis of cysteine (Salway 1999). Cystine declined ~43% across lactation, while methionine increased ~68%, possibly indicating that production of succinyl-CoA through methionine catabolism declined as lactation progressed and that conservation of methionine increased. The concentration of methionine within elephant seal milk decreases by ~10% across lactation, and if there is an increased need to conserve methionine with time fasting and lactating, reductions in milk constituency may reflect this need (Davis et al. 1995). Nevertheless, plasma methionine concentration in fasting and lactating elephant seals is up to 10 times lower than that measured in lactating and denning black bears (Wright et al. 1999), and elephant seal milk has been shown to contain the lowest concentrations of methionine reported in pinnipeds (Davis et al. 1995). In contrast to an increased need for methionine, the overall low concentration observed in milk and plasma may indicate a nominal methionine requirement for this species. This is further supported by comparison to studies on mammary extraction of methionine in cows and goats, where it is one of the most efficiently extracted AAs, approaching 80% extraction of the concentration in arterial blood supplying the mammary gland (Mepham 1982, 1987).

Glutamine is an active NH_4^+ scavenger formed from glutamate and NH_4^+ in muscle and liver cells, and it acts primarily to transport ammonia for urea synthesis in a nontoxic form. Lactating female elephant seals demonstrate a greater flux of urea with time fasting but do not demonstrate an increase in circulating levels of urea (Crocker et al. 1998). Provided that the increase in urea flux is primarily due to an increased rate of loss of NH_4^+ from glutamine, lower circulating glutamine concentrations should necessitate a compensatory increase in the rates of glutamine flux. Although not of a comparable magnitude, the observed increase in glutamic acid (glutamate) concentration could reflect increased deamination of glutamine and an alteration in the dynamics of glutamine-glutamic acid interconversion that occurs with diminishing nutrient availability.

Adult Males

Blood samples collected from adult male elephant seals were not collected at intervals similar to those for either lactating females or weanlings, and concentrations of FAAs cannot be compared to those groups. Concentrations of FAA should only be considered in the context of what is known of adult male elephant seal behavior and energy expenditure during the breeding season, much of which is qualitatively defined. Values of plasma FAA presented here should be used to guide future efforts investigating variation in plasma FAA concentration and flux with time fasting, combative behavior, and breeding effort.

Glutamine comprises the greatest fraction of FAAs in most terrestrial mammals, but in combative adult male elephant seals, alanine may be the most abundant FAA. Circulating alanine was 115% of the molar concentration of circulating glutamine in adult males, but timing of sampling in individual animals casts uncertainty on the comparability of the measures. Alanine is the primary AA precursor to glucose formation via gluconeogenesis and may play an important role in energy management, since adult males probably have requirements for the rapid mobilization of glucose (e.g., due to combat or the chasing of subordinate competitors). Intuitively, this suggests a greater reliance on protein stores to meet energy needs, a hypothesis that could be addressed by determining the rate at which isotopically labeled alanine is converted into glucose and gluconeogenic intermediates.

Collectively, the branched-chain amino acids (BCAAs; leucine, isoleucine, and valine) composed a substantial fraction of the total essential FAA concentration in adult male elephant seals. These AAs are important in the structuring of skeletal muscle, and all have glucogenic properties; for example, isoleucine and valine can function in anaplerotic reactions to insure continuation of Krebs cycle functionality (Salway 1999). Although possibly related to the content of prey consumed before the fast, high levels of both alanine and the BCAAs may suggest a potential requirement for these AAs that is dependent on the increased activity level, combative behavior, or reproductive effort of adult male elephant seals. Proximate causes

of high levels of free BCAAs range from the de novo reparation of tissue damaged during intrasexual competition to an increased need for glucose supporting exercise during the breeding season.

Summary

FAA profiles taken from lactating female and developing weanling elephant seals, both under the constraint of fasting, demonstrate notable differences in the circulating concentrations of specific FAAs. Reductions in protein catabolism experienced by fasting weanlings are likely mirrored in reductions of the total FAA pool, but developmental requirements should necessitate increases in the dedication of certain AAs to tissue synthesis. Reduction in the total FAA concentration of lactating elephant seals occurs in light of increased protein catabolism. Lactogenesis should impose modifications to nonlactating rates of AA synthesis and utilization, a restriction that may be mirrored by significant increases in select AA concentrations with time lactating. However, the impact of diminishing nutrient stores requires that AA flux measurements consider both metabolic requirements and milk synthesis needs in the interpretation of utilization rates. The sampling protocol used for adult males does not permit variations in FAA concentration to be compared to diminishing nutrient reserves, combative behavior, or breeding effort. However, values presented should be useful in guiding future investigations of AA dynamics within reproductively competitive adult males.

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