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THE PHYSIOLOGICAL TRANSITION FROM FASTING TO FEEDING IN WEANED ELEPHANT SEAL PUPS

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ABSTRACT

We studied energetics and food utilization in young elephant seals as they were first introduced to solid food following their long post-weaning fast. Using radioactive tracer techniques, we monitored changes in body composition, protein metabolism, and metabolic rate during fasting and initial feeding. In fasting animals, fat stores supplied nearly all energetic requirements. In feeding animals, 49% of protein ingested was retained as body tissue, allowing protein mass to increase. Body fat was lost at rates comparable to rates in fasting animals and continued to fuel the bulk of metabolism. Weight loss was arrested when animals consumed 786 g/d, or 40 kcal/$kg^{0.75}$/d, which was far less than their metabolic rates (63–206 kcal/$kg^{0.75}$/d). Surprisingly, the young seals were able to maintain weight and store protein while energy intake was below metabolic needs. This was possible because animals gained weight as water; they retained well-hydrated proteinaceous tissue while losing poorly-hydrated adipose tissue.

Key words: elephant seal, food utilization, food consumption, energetics.

Many pinnipeds undergo long fasts during their terrestrial breeding season. Elephant seals (*Mirounga angustirostris*), for example, abstain completely from food and water for 1–3 mo while breeding and then again while molting. Prior to a fast, an elephant seal's adipose stores can exceed 50% of its body weight (Ortiz *et al.* 1978, Costa *et al.* 1986), and during fasting fat oxidation provides the bulk of metabolic needs (Ortiz *et al.* 1978, Pernia *et al.* 1980). After fasting animals are visibly emaciated with few fat stores remaining (Costa *et al.* 1986). For elephant seals, life consists of a continuous cycle of tissue build-up, catabolism, and then recovery. In juveniles, this cycle seems especially arduous since development and growth must accompany periodic fasting and feeding. Our goal is to investigate the response to initial feeding following the post-weaning fast in young elephant seals.

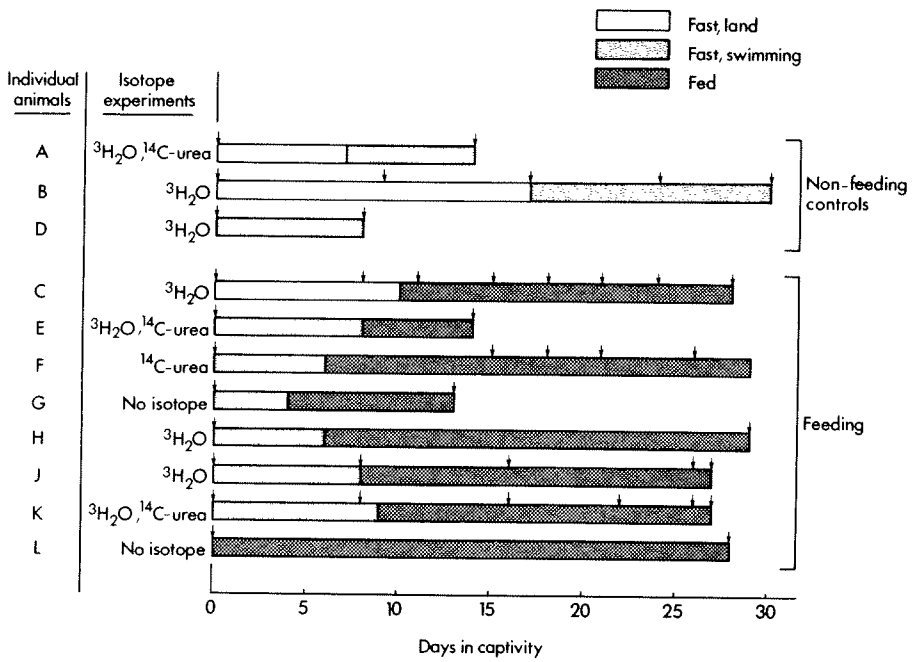

Figure 1. Experimental protocol for each of 11 animals used. The number of days each individual was kept fasting and feeding is represented by a bar. Weighing and blood sampling are indicated by arrows. Radioisotope experiments performed (water or urea) are indicated to the left of each bar.

Since feeding animals are inaccessible at sea, we brought naturally fasting weaned pups into captivity, offered them food, and found that they readily accepted it (Condit 1984). We measured body composition changes, weight changes, and protein utilization during fasting and feeding, asking the following questions: 1) When feeding begins, do young elephant seals continue to use their fat stores for energy or do they use the protein ingested? 2) Does body composition change when feeding begins? 3) How much food is needed to arrest and reverse weight loss as feeding begins?

Materials and Methods

Our studies were carried out at the University of California's Joseph Long Marine Laboratory in Santa Cruz. Eleven animals 6–12 wk of age, which had been fasted longer than 2 weeks, were transported from the Año Nuevo rookery to the lab. Each was kept in captivity for 2–4 wk. Seven were allowed to acclimate for 5–9 d before feeding, some in a dry enclosure with no water available and others in the same sea water tank in which they were subsequently fed (Fig. 1). The initial fast provided a baseline period of weight loss against which to compare feeding weight changes. Three animals were never fed and provided additional fasting controls, and a fourth was fed immediately after

capture (Fig. 1). After the acclimation period, we began offering dead anchovies, *Engraulis mordax,* and squid, *Loligo opalescens,* to the animals once or twice daily *ad libitum* (occasionally live fish of a variety of species were included). Feeding took place only while animals were swimming in sea water. All food was pre-weighed, and uneaten remains were collected and reweighed. Most fish were consumed promptly, often directly from a feeder's hand, so food consumption could be measured with minimum error.

Animals were weighed in a wooden box on a large platform balance. Average daily change in total body weight was expressed linearly by dividing weight loss by the duration of the experiment. For those animals which were fasted several days prior to feeding, weight loss was calculated during both periods. Appendix 1 gives details of weight loss calculations.

Changes in body composition, protein oxidation and retention, and metabolic rate were assessed using *in vivo* kinetics of radioactively labelled water and urea. The following measurements were made:

1) Body composition—Seven animals were used for measures of change in body composition, two fasting and five feeding. A single injection of ^3H_2O followed by a blood sample after equilibration allowed calculation of body water space (Ortiz *et al.* 1978). Adipose and lean tissue compartments were estimated from water space and body weight, assuming constant hydration values for each tissue (Yang *et al.* 1977, Ortiz *et al.* 1978). Adipose tissue is 10% water in elephant seals (Ortiz *et al.* 1978), and lean tissue is 73% water in a variety of mammals (Pace and Rathbun 1945). Body composition was calculated twice for each animal to measure the change of tissue compartments through time. Changes in body composition in feeding animals thus included a fasting acclimation period, which was about one third of the whole experiment (Fig. 1). Any error this might introduce in our estimates would be conservative, though, since we are seeking differences between feeding and fasting. Further details of body composition calculations are provided in Appendix 2.

We used rate of change of adipose and lean tissue compartments to estimate rates of protein retention and fat oxidation. For example, if an animal lost 600 g of adipose tissue per day, we estimated that fat was being oxidized at a rate of $0.9 \times 600 = 540$ g/d, since adipose tissue is 90% fat and 10% water. A similar calculation could be made for protein oxidation, assuming that protein comprised the entire solid fraction of lean tissue. Errors created by this assumption should be slight, and since we also measured protein oxidation using urea turnover (*see* below), major errors would be revealed. Calculations of changes in tissue compartment are elaborated in Appendix 2.

2) Protein consumption and oxidation—An independent estimate of the rate of protein oxidation was made in two fasting and two feeding animals using urea pool kinetics (*see* Pernia *et al.* 1980). A ^{14}C-urea injection was followed by two days of blood sampling to provide an estimate of urea turnover (*see* Appendix 3). Since protein oxidation (P_{ox}) and urea excretion are directly related, urea clearance can be used to calculate P_{ox} (Pernia *et al.* 1980).

Mean daily protein consumption (P_{con}) was taken from our measures of food intake and its protein content. We define protein retention (P_{ret}) as any protein

Table 1. Body weight, weight change and food intake in experimental animals. Animals are designated by the letters A–L, m = total body weight, I = food intake and MW = metabolic weight ($m^{0.75}$). Daily rate of weight loss is presented as an unadjusted figure (g/d), adjusted to body weight (%m/d, or percent of m), and adjusted to MW (g/MW/d). Time is the duration of the fasted or fed period, and subscripts refer to beginning (0) and end (t) of experiment. For fed animals, m_0 refers to weight when feeding began; when in parentheses these data were estimated using fasting loss in other animals (*see* Appendix 1).

					Daily rate of weight change		
Animal	Time (d)	m_0 (kg)	m_t (kg)	I (g/d)	(g/d)	(%m/d)	(g/MW/d)
Fasted:							
A	14	85.0	78.6	0	−459	−0.54	−16.3
B	30	127.7	111.8	0	−536	−0.42	−13.9
C	10	90.5	85.7	0	−434	−0.48	−14.7
D	8	119.1	113.6	0	−607	−0.51	−16.8
F	6	80.0	74.1	0	−984	−1.23	−36.8
J	8	92.1	81.6	0	−1,308	−1.42	−44.2
K	9	76.4	67.0	0	−1,047	−1.37	−40.5
Mean	12	95.8	87.5	0	−768	−0.85	−26.2
Fed:							
C	18	85.7	86.3	674	33	0.04	1.2
E	6	(73.0)	80.5	3,032	1,248	1.71	50.0
F	23	74.1	74.1	891	0	0.0	0.0
G	9	(72.8)	72.3	498	−55	−0.08	−2.2
H	23	(84.3)	85.5	715	51	0.06	1.8
J	19	81.6	76.8	450	−253	−0.31	−9.3
K	18	67.0	69.1	492	114	0.17	4.9
L	28	102.3	95.9	875	−225	−0.22	−7.0
Mean	18	80.1	80.1	953	114	0.17	4.9

consumed that was not oxidized, so P_{ret} is P_{con} minus P_{ox}. This estimate of P_{ret} can be compared to the one derived from changes in body composition, as described in the previous paragraph. Appendix 4 gives details of the calculations of P_{ret} and P_{ox}.

3) Metabolic rate—We estimated metabolic rate (MR) in each animal using tissue compartment changes. We assume any tissue loss (from body composition measurements) represents oxidation; by adding to this all components of food, we know total fat and protein oxidation. For example, if an animal lost 600 g of fat per day and consumed 100 g of protein (with no change in body protein component), then we assume its MR was derived solely from the oxidation of those two components. We use the standard caloric densities of fat and protein (fat yields 9.4 kcal/g and protein, 4.3 kcal/g, Schmidt-Nielsen 1983) to find total MR. We had two estimates of P_{ox} on which to base our calculation of MR, one based on body composition (section 1 above), the other on urea kinetics (section 2). They yielded similar values, and we present data based on the former.

Specific activities of injection solutions and blood samples were measured with

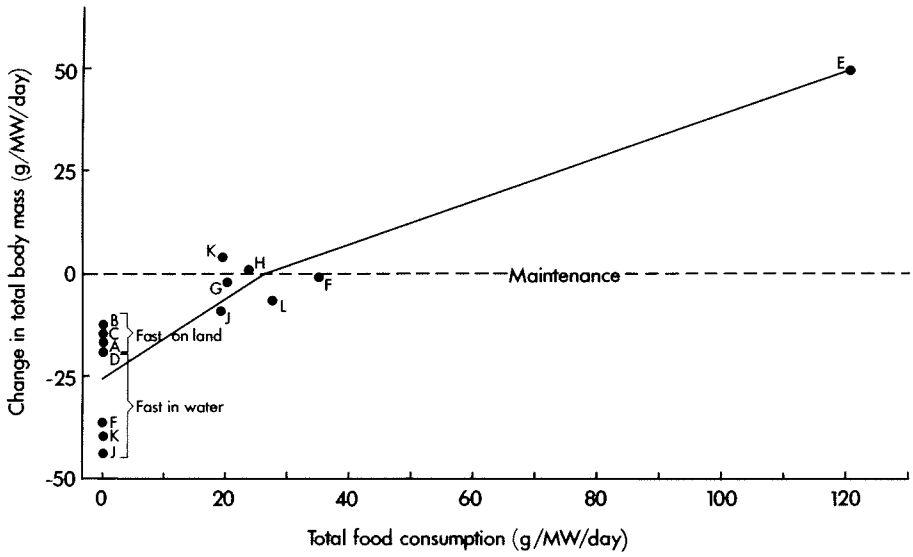

Figure 2. Weight change as a function of food consumption, both expressed relative to metabolic weight. Two separate regressions were done, one between zero food consumption and maintenance (excluding the point at the far right), the second from maintenance to maximum consumption (excluding the values for fasting weight loss). Regression to left of x-intercept: $y = 0.93x - 25.1$. x-Intercept = 26.9. Right regression: $y = 0.53x - 13.6$.

liquid scintillation on a Beckman LS-230 using conventional quench corrections when necessary. Blood urea concentration was determined using an Autoanalyzer. Water content of prey was estimated by freeze-drying diced samples to constant weight; anchovies were 75.1% water and squid 88.5% water. Protein content of fish (no squid were fed to seals during urea experiments) was taken from literature values (Watt and Merrill 1963, Eckert and Randall 1978); we used a value of 15.0 ± 1.2%, which holds for a wide variety of species. Fat composition was taken as 4% (Watt and Merrill 1963, Eckert and Randall 1978). The latter is highly variable, even among individuals within a species, but the amount of fat consumed in our experiments was small enough that the variability is of little consequence.

Results

Food Consumption and Body Weight Change

Mean weight loss in fasting animals was 768 ± 342 g/d. There was a marked bimodal distribution, with four animals losing 434–607 g/d, and three others losing 984–1,308 g/d (Table 1). This variation could not be accounted for by variation in body weight, since weight specific expressions were just as variable as absolute weight loss (Table 1).

Table 2. Body composition of experimental animals. N = water volume, m_l = lean mass, m_a = adipose mass (kg) and %a = adipose tissue as percent of total body weight. Subscripts 0 and t refer to beginning and end of experiment.

Animal	Time	N_0	N_t	m_{l0}	m_{lt}	m_{a0}	m_{at}	$\%a_0$	$\%a_t$
Fasted:									
A	14	52.3	50.1	69.6	67.2	15.4	11.4	18.1	14.5
B	30	57.2	51.7	70.6	64.4	57.1	47.3	44.7	42.3
Mean		55.8	50.9	71.1	65.8	39.5	29.4	35.7	29.0
Fed:									
C	28	38.3	38.8	46.5	48.0	44.0	38.3	48.6	44.4
E	14	54.0	56.1	73.1	76.4	7.4	4.1	9.2	5.1
H	29	39.1	44.0	47.8	56.4	42.7	29.1	47.2	34.0
J	27	39.6	38.8	48.3	49.5	43.8	27.3	47.6	35.5
K	27	40.1	36.8	51.6	47.5	24.8	21.6	32.5	31.3
Mean		39.6	39.9	49.2	51.1	37.1	26.0	38.2	30.4

Feeding animals consumed a mean of 953 ± 857 g/d (Table 1). Four gained weight while feeding, three lost weight, and one maintained weight, with the mean weight change for all being 114 ± 476 g gained/d. Animal E was very different from the others, consuming three times as much as any other animal (3,032 g/d, next highest 891) and gaining over 1,000 g/d (Table 1). Figure 2 shows a plot of weight change as a function of food intake.

Body Composition Changes

All animals except E started the experiment with extensive adipose stores (mean 38.2 ± 15.4% body weight). Animal E had less than 10% adipose tissue (Table 2).

During fasting, animals lost both lean and adipose tissue (mean loss, 189 and 307 g/d, respectively, Table 3). This includes loss of water, so actual tissue catabolized consisted of 276 g/d fat and 51 g/d lean components (mostly protein).

Feeding animals tended to gain body water and thus lean tissue while still losing adipose. In four of five experiments lean tissue mass increased, and the mean for all five was 96 g gained/d. All five feeding animals showed declines in adipose mass similar to those found in fasting animals (mean 351 g/d, Table 3).

Feeding led to an increase in fractional urea clearance (Table 4) and an increase in blood urea concentration (33.4 to 52.2 g/dl, $P < 0.002$, *F*-test). Blood urea concentration did not vary, however, with time since feeding. Total urea turnover and protein oxidation were higher in feeding animals but still represented a small fraction of total metabolism (Table 4 and *see* below). Since protein consumption exceeded protein oxidation (Table 4), animals were accumulating protein tissue at a rate of 54–95 g/d.

Table 3. Rate of change of tissue compartments and metabolic rate of experimental animals. r_l and r_a = rates of lean and adipose mass change, and MR = daily metabolic rate as calculated from tissue compartment change. Last column gives percent of total MR derived from protein oxidation; the remainder is from fat oxidation.

Animal	r_l (g/d)	r_a (g/d)	MR (kcal/d)	MR (kcal/MW/d)	% Protein to MR
Fasted:					
A	−171	−286	2,585	95	7.7
B	−207	−327	2,976	83	8.1
Mean	−189	−307	2,781	89	7.9
Fed:					
C	54	−204	2,093	74	10.5
E	236	−236	3,064	118	18.4
H	297	−468	4,193	150	0.5
J	44	−611	5,462	206	3.1
K	−152	−236	1,488	63	24.9
Mean	+96	−351	3,260	122	11.4

Estimates of protein retention based on urea turnover were reasonably similar to those based on lean tissue mass. The average from the urea turnover calculations was 75 ± 20 g protein/d in two animals, compared to 26 ± 48 g protein/d based on body composition measurements in five animals. Considering the magnitudes of the standard deviations, these two estimates cannot be said to disagree.

Metabolic Rate

Using body composition changes to estimate energy consumption yielded metabolic rates of 83 and 95 kcal/MW/d (MW = metabolic weight or

Table 4. Urea turnover (t.o., mean ± standard deviation of regression) and protein metabolism in experimental animals.

Animal	Urea t.o. (% per day)	Total urea pool (g)	r_u (g/d)	P_{ox} (g/d)	P_{con} (g/d)	P_{ret} (g/d)	P_{ret} (g/MW/d)
Fasted:							
A	32.6 ± 3.0	33.4	5.5	16.3	0	−16.3	−0.58
E	41.3 ± 6.2	33.4	6.8	19.7	0	−19.7	−0.73
Fed:							
F	130.2 ± 19	49.4	30.0	87.6	182.9	95.3	3.77
K	89.4 ± 23	63.6	21.9	63.9	118.1	54.2	2.31

weight $^{0.75}$) in the two fasting animals (Table 3). Estimates for the feeding animals (Table 3) were somewhat higher and quite variable (122 ± 58 kcal/MW/d).

Discussion

Our experimental animals maintained weight on food intake of 27 g/MW/d, equivalent to 786 g/d for a 90 kg animal, or less than 1% of its body weight. This is very low compared to food intake measured in other species of pinnipeds, which is generally 3–11% of body weight or 60–250 g/MW/d (Scheffer 1955, Depocas *et al.* 1971, Sergeant 1973, Jones 1981, Keiver *et al.* 1984, Ronald *et al.* 1984, Colleen Bates, pers. comm.). Because our animals expended a mean of 122 kcal/MW/d while feeding, it seemed paradoxical that weight could be maintained on energy intake as low as 35 kcal/MW/d (food intake was 27 g/MW/d; anchovies are 1.3 kcal/g, D. Costa, unpublished data). The body composition data resolved this apparent inconsistency. Animals were exchanging adipose tissue for lean tissue, and the latter is over seven times more hydrated than the former. Although the caloric content of their bodies declined, the animals gained enough water to balance the loss and hold their weight nearly constant.

Because all animals were fed *ad libitum,* the low food intake was voluntary. It seems unlikely that captive conditions were responsible for low rate of feeding, since the animals rarely appeared stressed and played with many fish for long periods. Instead, it appears that the high percentage of body fat inhibited their appetite, since animal E was the only one that ate enough to gain weight and had considerably fewer adipose stores than the others.

Besides maintaining weight, the animals were also able to spare protein on a calorically insufficient diet. Although we have ignored fecal nitrogen loss in our calculations, it is generally such a small proportion of ingested protein (6%, *see* Keiver *et al.* 1984 and Ronald *et al.* 1984) that our conclusion would be unaffected. In most mammals, a deficit in caloric intake forces protein catabolism, and nitrogen balance suffers (Munro 1964). Young elephant seals, however, were able to spare protein by utilizing their extensive lipid stores in place of exogenous sources of calories. Similarly, obese rats and mice on restricted diets conserve protein relative to normal animals (Longenecker and Sarett 1962, Marliss *et al.* 1974).

Since protein is not stored in non-functional depots like fats and carbohydrates, young seals must have been developing new muscle or other proteinaceous tissue. For a growing animal rich in fat stores but in need of lean tissue anabolism, this pattern of food utilization seems logical. Body fat is essential when fasting, but a burden when feeding at sea (depending on thermoregulatory needs, *see* Bryden 1964), so animals must eliminate adipose tissue and use exogenous protein for development. We speculate that this pattern of replacing adipose tissue with lean continues for the first year of a young elephant seal's life, since 10 mo old animals are no larger but are noticeably leaner than weaned pups. Although young elephant seals are literally weaned in 4 wk (Reiter *et al.* 1978),

they continue to live on calories from their mother's milk (stored in their adipose tissue) for much longer than this.

The increase in metabolic rate we observed when animals started feeding was probably real, although not statistically significant. All the animals were much more active when swimming (data in Condit 1984) than they were when hauled out, and fasting estimates included a haul out period (*see* Fig. 1). Higher activity and MR in water could explain the bimodal weight loss seen in Figure 2. The lowest rates of weight loss (434–536 g/d) were found in three animals which were held in the dry enclosure for a substantial part of their fast, whereas the remaining four values (607–1,308 g/d) represent weight loss while in the water. It is also possible that increased MR and weight loss when feeding could be attributed to specific dynamic action (SDA), which can be high on a protein diet (Krebs 1964, Gallivan and Ronald 1981).

An earlier estimate of MR in young elephant seals (Ortiz *et al.* 1978) was 167 kcal/MW/d in fasting animals. This is somewhat higher than our figure (89–122 kcal/MW/d), but given the conditions of our experiments, the difference is not surprising. Overall, our estimates of MR were variable, and we suggest more precise techniques for future studies, such as the use of doubly-labelled water (Lifson and McClintock 1966, Nagy and Costa 1980).

Despite variability in some of our quantitative estimates, we are secure in our qualitative conclusions. Two independent lines of evidence, one based on changes in body composition, the other on urea kinetics, led us to the same result: when young, fasting elephant seals begin feeding, they are able to retain a great deal of protein consumed while continuing to oxidize body fat for energy. Young seals store an enormous quantity of adipose tissue while nursing and then take advantage of it while they learn to swim and to eat, building protein tissue and maintaining weight on low food intake.

When elephant seals begin feeding at sea, lean tissue weight continues to increase while the blubber layer is reduced to sufficient thickness to provide adequate thermal insulation and buoyancy. In preparation for breeding and molting, however, adipose tissue stores must be increased to provide fuel for the 1–3 month fast. The physiological regulation of this annual cycle of fat deposition and recruitment and the regulation of body composition and buoyancy during development of young elephant seals pose intriguing subjects for future study.

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APPENDICES

Appendix 1. Calculation of Rate of Change of Total Body Weight (r_m)

Let m_0 = an animal's weight at the start of an experiment and m_t = weight at the end. Then

$$r_m = (m_t - m_0)/t. \quad \text{(A1-1)}$$

Most animals were weighed three times or more (Fig. 1), allowing separate estimates of r_m while fasting and feeding. In three animals weighed only twice, however, we had to use the estimate of weight loss during fasting from the other experiments, r_m(fast) to calculate weight when feeding began (time t1):

$$r_m(\text{fast}) = (m_{t1} - m_0)/t1. \quad \text{(A1-2)}$$

Then r_m(fed) in each of these 3 animals was found as

$$r_m(\text{fed}) = (m_{t2} - m_{t1})/(t2 - t1), \quad \text{(A1-3)}$$

with t2 being the time at the end of the experiment. These three animals thus generated independent estimates of feeding weight change but not of fasting weight change.

Appendix 2. Calculation of Body Water Space and Lean and Adipose Tissue Compartments

Let N = total body water, CPM = counts of ^3H_2O injected, and SA_0 = specific activity at equilibration (time 0), then

$$N = CPM/SA_0. \quad \text{(A2-1)}$$

Assume adipose tissue mass (m_a) is 10% water and lean tissue mass (m_l) is 73% water. By definition, m_a and m_l comprise the entire body weight (m) and hence all its water (N), so

$$N = 0.1\ m_a + 0.73\ m_l, \quad \text{(A2-2)}$$

$$m = m_a + m_l. \quad \text{(A2-3)}$$

These can be solved for m_a and m_l:

$$m_l = 1.59\ N - 0.159\ m, \quad \text{(A2-4)}$$

$$m_a = 1.159\ m - 1.59\ N. \quad \text{(A2-5)}$$

We express changes in compartments linearly:

$$r_l = [m_l(t) - m_l(0)]/t, \quad \text{(A2-6)}$$

$$r_a = [m_a(t) - m_a(0)]/t, \quad \text{(A2-7)}$$

where r_l and r_a are average daily rates of change of lean and adipose tissue mass.

Finally, we use the hydration values of each compartment to determine actual tissue loss:

$$P_{ret} = r_l \times 0.27, \quad \text{(A2-8)}$$

$$F_{ox} = r_a \times 0.90. \quad \text{(A2-9)}$$

P_{ret} = total protein tissue retained (possibly a negative number) and F_{ox} = total fat lost by the animal. In a fasting animal, P_{ret} is the same as protein oxidation (P_{ox}). For a feeding animal, P_{ox} can be calculated from P_{ret} by knowing total protein consumption (see Eq. A4-4 below).

Appendix 3. Calculation of Turnover Kinetics

The general equation relating turnover of a single compartment pool to the specific activity (SA at time t) of an injected tracer is

$$SA_t = SA_0 \times \exp[-\int (r_{tot}/N) \times dt], \quad \text{(A3-1)}$$

where r_{tot} = total turnover (actually influx) and N is the pool size. As long as turnover is a constant proportion of volume (r/N constant), Eq. A3-1 becomes a simple exponential decay, and fractional turnover (r/N) is the slope of a plot of $\ln(SA_t)$ versus time. Providing N is known, total turnover is easily derived. We used computer simulated experiments to examine the consequences of varying r/N; without failure, an estimate of r based on Eq. A3-1 accurately reflects the true average value, even if there is high day to day variation in r.

Appendix 4. Calculation of Urea Kinetics and Protein Oxidation and Retention

Decline in specific activity of ^{14}C-urea in blood yielded fractional urea turnover (Eq. A3-1). To find a value for urea pool size (N_u), we used blood urea concentration (BUC) and total water volume (N) from eq. A1-1:

$$N_u = BUC \times N. \quad \text{(A4-1)}$$

This allowed us to find total urea turnover (r_u) using Eq. A3-1.

We found protein oxidation rate directly from r_u:

$$P_{ox} = 2.92 \times r_u \quad \text{(A4-2)}$$

and we calculated mean daily protein consumption as

$$P_{con} = I \times \%P, \quad \text{(A4-3)}$$

where I = daily food intake and %P = proportion of protein in the diet. Protein retention is the difference between consumption and oxidation,

$$P_{ret} = P_{con} - P_{ox}. \quad \text{(A4-4)}$$

This value of P_{ret} can be compared to the one calculated from body composition change in Eq. A2-8.

Appendix 5. Calculations of Metabolic Rate

Metabolic rate is generated entirely by the oxidation of fats and proteins in these animals, and since we know the rate of oxidation, we can easily find MR:

$$MR = 9.4 \times (F_{ox} + F_{con}) + 4.3 \times P_{ox}, \quad \text{(A5-1)}$$

where F_{con} represents any fats consumed in food, and 9.4 and 4.3 are stoichiometric constants for the number of kcal produced by oxidation of fat and protein respectively (Schmidt-Nielsen 1983). F_{ox} comes from Eq. A2-9, P_{ox} from either Eq. A2-8 or A4-2.

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