INVITED PERSPECTIVES IN PHYSIOLOGICAL AND BIOCHEMICAL ZOOLOGY

# Evolution of Regulatory Responses to Feeding in Snakes

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Accepted 10/1/99

**ABSTRACT**

Do animal species that normally consume large meals at long intervals evolve to down-regulate their metabolic physiology while fasting and to up-regulate it steeply on feeding? To test this hypothesis, we compared postfeeding regulatory responses in eight snake species: four frequent feeders on small meals and four infrequent feeders on large meals. For each species, we measured factorial changes in metabolic rate, in activities and capacities of five small intestinal brush border nutrient transporters, and in masses of eight organs that function in nutrient processing after consumption of a rodent meal equivalent to 25% of the snake's body mass. It turned out that, compared with frequent feeders, infrequent feeders digest that meal more slowly; have lower metabolic rates, organ masses, and nutrient uptake rates and capacities while fasting; have higher energy expenditure during digestion; and have higher postfeeding factorial increases in metabolic rate, organ masses, and nutrient uptake rates and capacities. These conclusions, which conform to the hypothesis mentioned above, remain after phylogeny has been taken into account. The small organ masses and low nutrient transporter activities during fasting contribute to the low fasting metabolism of infrequent feeders. Quantitative calculations of partial energy budgets suggest that energy savings drive the evolution of low mass and activities of organs during fasting and of large postfeeding regulatory responses in infrequent feeders. We propose further tests of this hypothesis among other snake species and among other ectotherms.

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*Physiological and Biochemical Zoology* 73(2):123–141. 2000.  1522-2152/2000/7302-9930$03.00

## Introduction

A central goal of evolutionary physiology is to elucidate how physiological systems and the natural variation in their performances have evolved (Huey 1987; Garland and Carter 1994; Bennett 1997). Because many physiological traits are essential to survival, growth, and reproductive success, they are likely to be under strong selection pressure. Hence, comparative physiologists now seek to identify not only the molecular and cellular mechanisms proximately responsible but also the evolutionary history and selective forces ultimately responsible for interspecific differences. This approach has been applied in the field of digestive physiology because digestive mechanisms have obvious consequences for features of whole-animal performance, such as feeding ecology, nutrition, energy budgets, and digestive efficiency (Karasov and Diamond 1988; Hammond et al. 1994; Martínez del Rio et al. 1995; Hume and Biebach 1996; Karasov and Hume 1997; Witmer 1998). Examples include the correlations of a species' intestinal nutrient transport rates (Karasov and Diamond 1988) and hydrolytic enzyme activities (Hernandez and Martínez del Rio 1992) with its dietary habits (e.g., carnivore vs. herbivore) and of its up-regulation of intestinal performance with its increased food intake during lactation or cold exposure (Hammond and Diamond 1994; Konarzewski and Diamond 1994).

One recurring finding in studies of the usual mammalian model species (such as mice and rats) is that intestinal nutrient transporter activities are regulated only over a narrow span, generally not exceeding a factor of 2 from minimum to maximum activity (Ferraris and Diamond 1989). Did those narrow spans evolve in response to the modest variation in daily demand encountered in nature by the intestines of those species that normally consume small meals frequently (such that the intestine usually contains some food)? We hypothesized that larger regulatory spans might characterize species that in nature feed infrequently on large meals, thereby imposing large variations in demand on their intestines. Such animal candidates would include sit-and-wait-foraging snakes that feed in the wild, at intervals exceeding 1 mo, on prey equivalent to 25%–160% of the snake's body mass (Pope 1961; Greene 1997).

This article tests this evolutionary hypothesis by comparative physiological studies of eight snake species: four species that in the wild consume small meals at frequent intervals and four species that consume large meals at long intervals. For each species we measured the factorial postfeeding changes in metabolic rate, in activities of five small intestinal brush border

nutrient transporters, and in masses of the small intestine and seven other organs that function in nutrient processing, following consumption of a rodent meal equivalent to 25% of the snake's body mass. Our goals were (*a*) to test the hypothesis that differences in physiological responses are correlated with differences in feeding habits, (*b*) to test whether such a correlation is independent of phylogeny, and (*c*) to test quantitatively an economic model of the conditions necessary for the evolution of physiological regulation.

## Material and Methods

### *Snake Species and Their Feeding Habits*

All eight snake species studied include small mammals in their natural diet (thereby enabling us to use standardized rodent meals), but they differ greatly in natural meal size, feeding frequency, and foraging method. Our information about natural feeding habits came from published studies and our own and others' observations (Wall 1912; Hamilton and Pollack 1956; Pope 1961; Greene 1983; Platt 1984; Secor and Nagy 1994; Plummer and Congdon 1996; J. Rodriguez-Robles, personal communication). This information permitted us to select four species that feed at long intervals of 4–6 wk: the rosy boa *Lichanura trivirgata* and the boa constrictor *Boa constrictor* of family Boidae, the Burmese python *Python molurus* of family Pythonidae, and the sidewinder rattlesnake *Crotalus cerastes* of family Viperidae. We also selected four species that feed at short intervals of 1–2 wk: the racer *Coluber constrictor*, the kingsnake *Lampropeltis getula*, the coachwhip *Masticophis flagellum*, and the bullsnake *Pituophis melanoleucus* of family Colubridae. Our estimates of the natural feeding intervals of these snakes as adults are summarized in Figure 8.

These eight species exhibit differences in foraging tactics, natural diet, distribution, and habitat, independent of the dichotomy in feeding interval. *Boa constrictor* and *P. molurus* hunt mammals and birds by night or day by ambush, but the former's geographical range is Neotropical, while the latter's is tropical Southeast Asia (Pope 1961; Greene 1983). *Lichanura trivirgata* ambushes or actively searches for small mammals during daylight or at night (Wright and Wright 1957). *Crotalus cerastes* ambushes small mammals and lizards by day or night (Secor 1995*b*). *Lichanura trivirgata*, *C. cerastes*, *C. constrictor*, *L. getula*, *M. flagellum*, and *P. melanoleucus* are native to North America, with *L. trivirgata* and *C. cerastes* confined to brushlands and deserts of the Southwest (*C. cerastes* in loose-sand habitats) and the latter four species having wider geographical ranges (Stebbins 1985; Conant and Collins 1991). Of the latter four species, *L. getula* and *P. melanoleucus* search for prey in the morning and late afternoon (*P. melanoleucus* seeking mammals and birds and bird eggs; *L. getula*, mammals, birds, lizards, and snakes), while *C. constrictor* and *M. flagellum* search throughout the day, on or below the ground and through vegetation, for mammals, birds, snakes, lizards, and insects (Imler 1945; Hamilton and Pollack 1956; Fitch 1982; Platt 1984; Conant and Collins 1991; Secor 1995*b*).

The *B. constrictor* and *P. molurus* used in our study were captive-born juveniles 1–2 yr old, purchased from commercial breeders. The other six species used were subadults and adults collected from the wild (*L. getula*, *C. cerastes*, and *L. trivirgata* in California; *M. flagellum* and *P. melanoleucus* in Texas; and *C. constrictor* in Florida). We housed snakes individually in plastic boxes (40 × 25 × 15 cm) at 30°C under a light : dark photoperiod of 14L : 10D. Snakes were fed biweekly meals of laboratory mice or young rats (depending on snake size), with water available ad lib. To ensure that snakes were completely postabsorptive before measuring responses to feeding, we withheld food from snakes for one month.

In this study, we used 23 individuals of *P. molurus*, 21 *M. flagellum*, 19 *C. cerastes*, 18 *P. melanoleucus*, eight *C. constrictor*, and six each of the other three species. We reported previously postfeeding responses for 18 of the 19 *C. cerastes* and for all *P. molurus* (Secor et al. 1994; Secor and Diamond 1995, 1997*b*). Metabolic rates before and after feeding were measured in five individuals of each species. We measured digestion rates, intestinal nutrient uptake rates, and organ masses in six to 18 individuals of each species, killed by severing the spinal cord immediately posterior to the head. Of those six to 18 individuals, many of which had been used previously to measure metabolic response, three of each species were killed in the fasting state (after the 30-d fast), while the remaining three to 15 individuals of each species were killed at 1–5 time points after consuming a rodent meal (laboratory mice or rats) equaling 24.9% ± 0.2% of the snake's fasted body mass. Fed snakes were killed in sets of three at 0.5, 1, 3, 6, and 14 d postfeeding for *M. flagellum*, *C. cerastes*, and *P. molurus*; at 0.5, 1, 3, and 6 d postfeeding for *P. melanoleucus*; and at 1 d postfeeding for the other four species. Hence, we compared responses over a complete bout of digestion for four species (two frequent and two infrequent feeders) and responses 1 d after ingestion for all eight species.

### *Rates of Digestion*

We assessed rates of digestion from the percentage of the ingested meal mass remaining within the stomach and small intestine of fed snakes. Stomachs and small intestines were each weighed on removal from the snake, emptied of their contents, and reweighed. The difference between the full and empty weights was recorded as the wet mass of the organ content. Organ content wet mass was divided by meal wet mass to express the relative extent of digestion.

### *Metabolic Rate*

We assessed metabolic rates of individual snakes as oxygen consumption rates ($\dot{V}O_2$ in units of mL $g^{-1}$ $h^{-1}$) at 30°C, mea-

sured by closed-system respirometry as previously described (Secor and Diamond 1997*b*, based on Vleck 1987). The $\dot{V}O_2$ of snakes fasted for 30 d was measured daily at 0600–0900 hours for 4 or 5 d. Each snake's lowest value of $\dot{V}O_2$ during those days was taken as its standard metabolic rate (SMR). On the following morning, snakes were weighed and fed the rodent meal equivalent to 25.0% ± 0.1% of the snake's body mass. We continued measurements of $\dot{V}O_2$ at 12-h intervals (at 0800 and 2000 hours) for 2–3 d and then (as $\dot{V}O_2$ declined) once daily at 0800 hours for 6–11 d.

For each snake we quantified the following eight parameters of their metabolic response, discussed in more detail by Secor and Diamond (1997*b*): (1) SMR in units of mL $g^{-1}$ $h^{-1}$; (2) SMR in units of mL $h^{-1}$, allometrically corrected (by a mass exponent of 0.7; see Andrews and Pough 1985 and Chappell and Ellis 1987) to a body mass of 350 g (the mean mass of these 40 snakes was 352 ± 46 g); (3) postfeeding peak $\dot{V}O_2$ (mL $g^{-1}$ $h^{-1}$); (4) postfeeding peak $\dot{V}O_2$ (mL $h^{-1}$) allometrically corrected (by a mass exponent of 0.9; see Secor and Diamond 1997*b*) to a body mass of 350 g; (5) factorial scope of peak $\dot{V}O_2$ (i.e., ratio of peak $\dot{V}O_2$ to SMR); (6) duration of $\dot{V}O_2$ significantly elevated above fasting levels; (7) specific dynamic action (SDA; additional energy expended on digestion, assimilation, and biosynthesis) quantified (as kJ $kg^{-1}$) from the extra $O_2$ consumed above SMR during the period of significantly elevated $\dot{V}O_2$; and (8) SDA as a percentage of the energy content of the meal. In calculating the last two variables, we assumed an energy conversion of 19.8 J expended per milliliter $O_2$ consumed and an energetic value of 8 kJ $g^{-1}$ wet mass for the rodent meals (Gessaman and Nagy 1988; Secor and Diamond 1995).

### *Intestinal Nutrient Uptake*

We measured nutrient uptake rates across the intestinal brush border membrane in vitro by the everted sleeve technique, as described in detail by Secor and Diamond (1997*a*), based on Secor et al. (1994) and Karasov and Diamond (1983). We used five nutrients to probe five different brush border transporters: the sugar D-glucose, to probe the intestinal brush border $Na^+$-coupled glucose transport protein SGLT1 and the amino acids L-aspartic acid, L-leucine, L-lysine, and L-proline to probe, respectively, the acidic amino acid, neutral amino acid, basic amino acid, and imino acid transporters. As previously explained (Secor et al. 1994; Secor 1995*a*; Secor and Diamond 1995), we report for each snake species the nutrient uptake rates (nmol $min^{-1}$ $mg^{-1}$) from the anterior two-thirds of the small intestine and from the distal one-third of the small intestine and the total nutrient uptake capacity (expressed as $\mu$mol $min^{-1}$) of the small intestine, calculated as the summed products of regional wet masses times regional nutrient uptake rates.

To assess the effects of feeding on organ masses, we weighed the wet masses of the heart, paired lungs, liver, empty stomach, pancreas, empty small intestine, empty large intestine, and paired kidneys immediately on their removal from the snake. All organs except the small intestine were oven dried (60°C) and reweighed. We calculated the dry mass of the total small intestine as the summed product of regional intestinal length times the dry mass of a 1-cm sleeve removed from each intestinal region.

### *Statistical Analysis*

For each species, we used a repeated-design ANOVA to test for significant effects of time (before and after feeding) on $\dot{V}O_2$. We employed a one-way ANOVA to test for each species the effects of sampling time on intestinal uptake rates and for species effects on rates of digestion, metabolic variables, and normalized (i.e., divided by body mass) uptake capacities and organ masses (Zar 1974). Differences among sampling times in nonnormalized intestinal uptake capacities and organ masses were tested by ANCOVA, with body mass as the covariate. For the few instances where there was a significant interaction between body mass and sampling time, we report ANOVA results. In conjunction with ANOVAs and ANCOVAs, we made post hoc pairwise mean comparisons to identify any significant distinctions between pairs of time samples and between pairs of species. We report throughout the text the results of ANOVAs and ANCOVAs in terms of their $F$ and $P$ values, and we report the results of significant pairwise comparisons as their $P$ values. We designate statistical significance as $P \leq 0.05$ and report mean values or mass-adjusted means (least square means from ANCOVA) ± 1 SEM (standard error of the mean). All statistical analyses were conducted by the microcomputer version of SAS (SAS Institute 1988).

### *Phylogenetic Statistical Analysis*

This article seeks to identify physiological traits that have evolved in association with feeding habits (natural meal size and meal interval). But phylogeny can be another important determinant of trait variation. Because species have descended hierarchically from ancestors and thus share evolutionary histories to differing degrees, traits compared among different species cannot be considered as unconstrained independent variables. For example, our four species of frequent feeders all belong to the family Colubridae, while our four species of infrequent feeders all belong to three other families. How can we assess whether any trait variation that we observed is really correlated with feeding interval and is not just a rigidly inherited characteristic distinguishing species of Colubridae from species of other snake families?

We addressed this question by using the method of phylogenetically independent contrast analysis (Felsenstein 1985) to determine whether there exists a significant phylogenetically

corrected correlation between feeding habits (quantified as feeding interval) and the magnitudes of postfeeding responses (see Garland et al. [1992] and Losos and Miles [1994] for the rationale of this approach). This technique uses phylogenetic relationships and divergence times between taxa to transform values measured from $N$ species into $(N-1)$ contrasts. Because each contrast is a comparison between sister taxa or nodes along successive bifurcations of a phylogenetic tree, the contrasts are statistically independent and can be analyzed by conventional statistical methods. We performed this analysis on five postfeeding response variables for which we had especially extensive and accurately measurable data: SDA in units of kJ $kg^{-1}$, factorial peak in $\dot{V}O_2$, and the 1-d-postfeeding factorial increases in intestinal uptake capacity of D-glucose, L-leucine, and L-proline. Because there is currently no published phylogeny that includes the eight snake species of this study, we constructed a hypothetical phylogeny with estimated branch lengths (to be presented in Fig. 8) from published phylogenies, including at least one of our species or a congener (Dowling et al. 1983; Dessauer et al. 1987; Rage 1987; Kluge 1991; Heise et al. 1995), and from unpublished recommendations by D. Cundall, H. Dowling, and H. Greene. We log-transformed the four variables and standardized each independent contrast by its standard deviation (square root of the sum of branch lengths) to achieve equal weighting (Garland et al. 1992). The computation of standardized contrast values and of their correlation coefficients was performed by the computer program PDAP (Phenotypic Diversity Analysis Programs; Garland et al. 1993).

## Results

### *Digestion Rates*

We assessed digestion rate from the mass of the contents of the stomach and small intestine as a percentage of ingested meal mass at various times after ingestion (Fig. 1). Data for all eight species are available at 1 d postfeeding. At that time the stomach (Fig. 1*A*) had broken down and passed 5%–36% of the ingested mass, but the four frequently feeding species had by then passed a greater percentage ($P = 0.017$) of the ingested meal (30% ± 3%) than had the four infrequent feeders (15% ± 4%). In addition, data are available for four species at 12 h and 1, 3, 6, and 14 d postfeeding. At those times digestion had progressed further for both species of frequent feeders than for either infrequent feeder (Fig. 1*B*). For instance, at 12 h 7% and 9% of the meal mass had passed out of the stomach and small intestine of the frequent feeders *Masticophis flagellum* and *Pituophis melanoleucus*, which was significantly greater ($P < 0.04$) than the mere <1% reduction of stomach contents of the infrequent feeders *Crotalus cerastes* and *Python molurus* (Fig. 1*B*). All snake species had completely emptied their stomach and small intestine by 30 d postfeeding. Thus, even for meals of the same relative size, frequently feeding species digest the meal more rapidly than do infrequently feeding species.

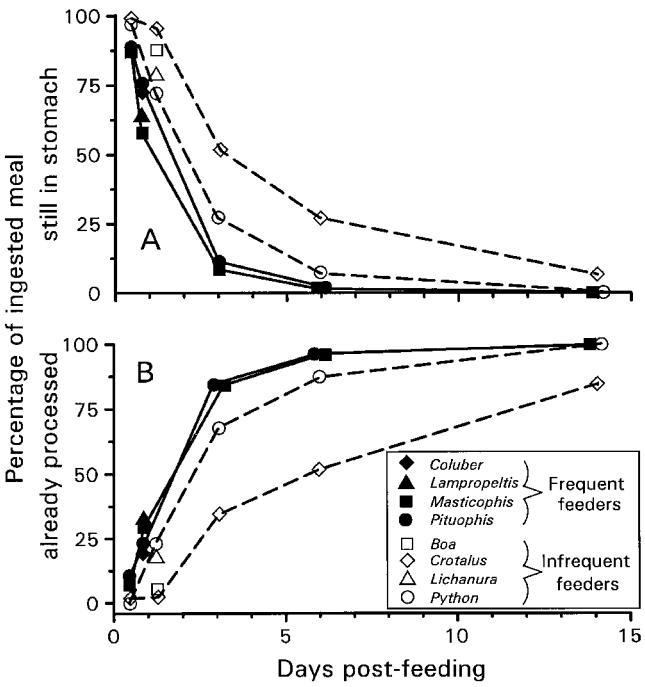



Figure 1. Percentage of ingested meal that was recovered as stomach contents (*A*) and percentage that had passed through both the stomach and small intestine (*B*; calculated as total meal mass minus stomach and small intestinal contents) as a function of days postfeeding for eight snake species. Solid symbols represent frequently feeding species, while open symbols represent infrequently feeding species. All snakes had consumed meals equaling 25% of their body mass. Percentages were measured at 0.5, 1, 3, 6, and 14 d postfeeding for four snake species (*Crotalus cerastes*, *Masticophis flagellum*, *Pituophis melanoleucus*, and *Python molurus*) but only at 1 d postfeeding for the other four species ($n = 3$ for each sampling period). Symbols are occasionally offset to avoid overlapping. Note that the frequently feeding species experience faster rates of passage than the infrequently feeding species.

### *Metabolic Response*

All eight species experienced stable $\dot{V}O_2$ rates during the 4–5 d of fasting measurements (temporal coefficient of variation = 7.4% ± 1%). Standard metabolic rates (SMR) differed significantly among the eight species when calculated either as milliliters per gram per hour ($F_{7,32} = 63.0$, $P \leq 0.0001$) or else as milliliters per hour, adjusted allometrically to a body mass of 350 g ($F_{7,32} = 33.4$, $P \leq 0.0001$; Table 1). In pairwise comparisons, all four frequently feeding species possessed significantly ($P < 0.036$) greater SMRs than any of the four infrequently feeding species. Averaged together, SMR of the four frequent feeders was 2.2-fold greater than the average SMR of the four infrequent feeders.

As illustrated in Figure 2, $\dot{V}O_2$ of each snake increased rapidly within 24 h after feeding, peaked, and then declined more slowly. Qualitatively similar profiles of postfeeding metabolism

Table 1: Body size, meal size, standard metabolic rate (SMR), and postfeeding metabolic measures (peak $\dot{V}O_2$, scope of peak $\dot{V}O_2$, duration, and SDA) of four frequently feeding and four infrequently feeding snake species

| | Frequent Feeders | | | | Infrequent Feeders | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | *Coluber constrictor* | *Lampropeltis getula* | *Masticophis flagellum* | *Pituophis melanoleucus* | *Boa constrictor* | *Crotalus cerastes* | *Lichanura trivirgata* | *Python molurus* | ANOVA |
| Body mass (g) | 223 ± 31 | 188 ± 18 | 273 ± 32 | 732 ± 235 | 346 ± 48 | 161 ± 16 | 163 ± 24 | 736 ± 81 | $P \leq .0001$ |
| Meal size (% of body mass) | 25.0 ± .3 | 24.8 ± .3 | 25.1 ± .2 | 25.1 ± .3 | 25.1 ± .1 | 25.0 ± .3 | 25.0 ± .2 | 25.0 ± .07 | $P = .979$ |
| SMR (mL $g^{-1}$ $h^{-1}$) | .098 ± .005$^{D}$ | .063 ± .004$^{C}$ | .054 ± .004$^{BC}$ | .047 ± .002$^{B}$ | .036 ± .002$^{A}$ | .029 ± .001$^{A}$ | .027 ± .002$^{A}$ | .032 ± .002$^{A}$ | $P \leq .0001$ |
| SMR (mL $h^{-1}$) adjusted to 350 g | 34.6 ± 3.4$^{F}$ | 18.2 ± 1.4$^{E}$ | 17.3 ± 1.4$^{DE}$ | 19.8 ± 1.1$^{e}$ | 12.0 ± .4$^{BC}$ | 7.9 ± .6$^{AB}$ | 7.2 ± .4$^{A}$ | 13.7 ± 1.0$^{c,d}$ | $P \leq .0001$ |
| Peak $\dot{V}O_2$(mL $g^{-1}$ $h^{-1}$) | .50 ± .02$^{C}$ | .44 ± .03$^{BC}$ | .44 ± .03$^{AB}$ | .37 ± .02$^{AB}$ | .66 ± .06$^{D}$ | .29 ± .02$^{A}$ | .42 ± .01$^{BC}$ | .57 ± .05$^{CD}$ | $P \leq .0001$ |
| Peak $\dot{V}O_2$ (mL $h^{-1}$) adjusted to 350 g | 157 ± 6$^{C}$ | 143 ± 9$^{BC}$ | 117 ± 7$^{AB}$ | 133 ± 7$^{BC}$ | 220 ± 21$^{D}$ | 94 ± 5$^{A}$ | 135 ± 4$^{BC}$ | 213 ± 18$^{d}$ | $P \leq .0001$ |
| Scope of peak $\dot{V}O_2$ (peak $\dot{V}O_2$/SMR) | 5.4 ± .4$^{A}$ | 7.0 ± .1$^{A}$ | 5.9 ± .7$^{A}$ | 8.0 ± .4$^{AB}$ | 18.5 ± 1.9$^{C}$ | 9.9 ± .2$^{B}$ | 15.9 ± 1.3$^{C}$ | 17.7 ± 1.3$^{C}$ | $P \leq .0001$ |
| Duration (d) | 4 | 4 | 5 | 5 | 8 | 12 | 9 | 8 | … |
| SDA (kJ $kg^{-1}$) | 309 ± 20$^{AB}$ | 298 ± 35$^{AB}$ | 258 ± 28$^{A}$ | 288 ± 16$^{A}$ | 670 ± 40$^{E}$ | 455 ± 9.9$^{C}$ | 357 ± 15$^{B}$ | 581 ± 26$^{D}$ | $P \leq .0001$ |
| SDA (% of ingested kJ) | 15 ± 1$^{AB}$ | 14 ± 2$^{A}$ | 13 ± 1$^{A}$ | 14 ± 1$^{A}$ | 33 ± 2$^{D}$ | 21 ± 1$^{C}$ | 18 ± 1$^{BC}$ | 30 ± 1$^{D}$ | $P \leq .0001$ |

Note. Metabolic variables are described in the text. Entries are mean values ± 1 SE. Sample size is five individuals for each species. For each metabolic variable, superscript letters that are shared denote nonsignificant ($P > 0.05$) differences between means as determined from planned pairwise comparisons.

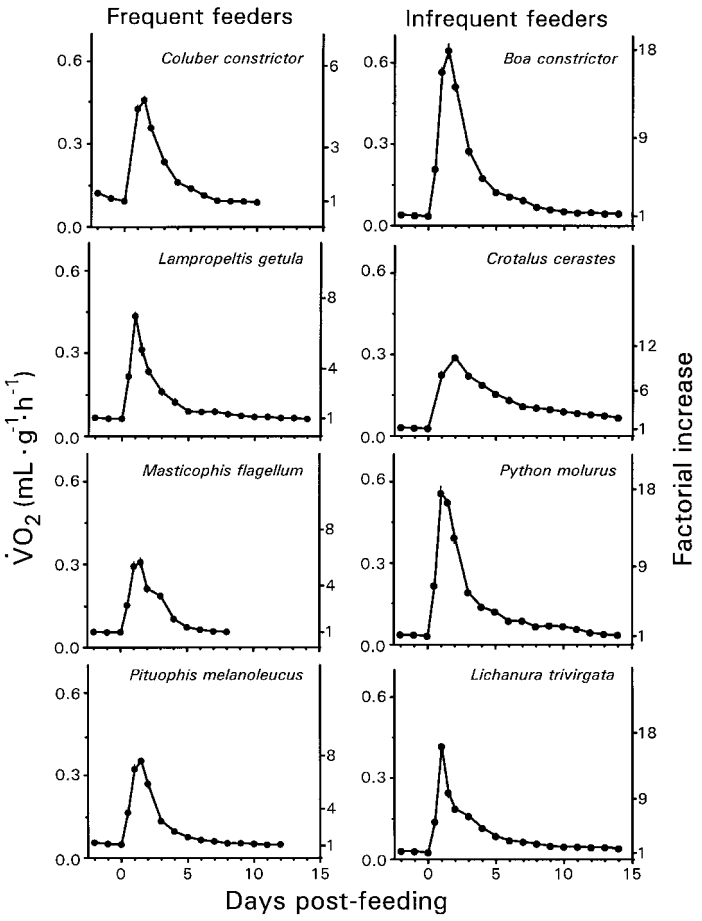



Figure 2. Mean oxygen consumption rates (left-hand ordinate scale) of eight species of snakes ($n = 5$ for each species) for 3 d before and up to 14 d after the consumption of rodent meals equaling 25% of the snake's body mass. The right-hand ordinate scale gives the factorial increase in $\dot{V}o_2$ above prefeeding values. In this and subsequent figures, vertical bars represent $\pm 1$ SE and are omitted if SE is smaller than the symbol for mean value. Note that the four frequently feeding species have higher prefeeding (fasting) $\dot{V}o_2$ but experience only a five- to sevenfold increase in $\dot{V}o_2$ on feeding, whereas the four infrequently feeding species have lower fasting $\dot{V}o_2$ but undergo a 10- to 18-fold increase in postfeeding $\dot{V}o_2$.

are exhibited by other species ranging from fish to mammals (Brody 1945; Jobling 1981; Janes and Chappell 1995). Postfeeding peaks in $\dot{V}o_2$ were reached at 24 h postfeeding for *Lampropeltis getula*, *Lichanura trivirgata*, and *P. molurus*; at 36 h for *Boa constrictor*, *Coluber constrictor*, *M. flagellum*, and *P. melanoleucus*; and at 48 h for *C. cerastes*. Heights of peaks in $\dot{V}o_2$ differed significantly among the eight species when expressed either as milliters per gram per hour ($F_{7,32} = 14.7$, $P \leq 0.0001$) or as milliliters per hour, adjusted allometrically to a body mass of 350 g ($F_{7,32} = 10.2$, $P \leq 0.0001$; Table 1). Postfeeding peaks in $\dot{V}o_2$ were significantly ($P < 0.02$) higher for the infrequent feeders *B. constrictor* and *P. molurus* than for the other six species.

The factorial scope of peak $\dot{V}o_2$ (peak $\dot{V}o_2$/SMR) differed significantly ($F_{7,32} = 30.3$, $P \leq 0.0001$) among the eight species, ranging from only $5.4 \pm 0.4$ for the frequent feeder *C. constrictor* to $18.5 \pm 1.9$ for the infrequent feeder *B. constrictor* (Fig. 2; Table 1). The peak factorial scope averaged for the four infrequent feeders was 2.4 times the scope averaged for the four frequent feeders. Peak scopes of each infrequent feeder exceeded values for all four frequent feeders, and values for three of the infrequent feeders (all except *C. cerastes*) were significantly higher ($P < 0.0002$) than values for any of the other five species. The four frequent feeders maintained significantly elevated $\dot{V}o_2$ for 4–5 d after feeding, which was only half of the duration (8–12 d) of elevated $\dot{V}o_2$ experienced by the four infrequent feeders (Table 1).

The extra energy expended during digestion (SDA) varied significantly among snake species when quantified either as kilojoules expended per kilogram of body mass ($F_{7,32} = 7.85$,

$P \leq 0.0001$) or as a percentage of ingested energy ($F_{7,32} = 43.6$, $P \leq 0.0001$; Table 1). For either measure of SDA, values for three infrequent feeders (all except *L. trivirgata*) were significantly greater ($P < 0.004$) than values for any of the four frequent feeders. Averaged among the four infrequent feeders, SDA was 1.8-fold greater than the average SDA for the four frequent feeders.

Thus, infrequent feeders have lower fasting metabolic rates but higher metabolic scopes on feeding, longer durations of elevated metabolism on feeding, and higher total extra energy expenditure on feeding (SDA) than do frequent feeders.

### *Intestinal Nutrient Uptake*

Figure 3 illustrates the postfeeding profiles of L-leucine, L-proline, and D-glucose uptake by the anterior small intestine for the four snake species studied at different times after feeding. The two infrequent feeders both experienced significant postfeeding increases ($P < 0.001$) in uptake for all three nutrients, maintained those elevated uptake rates during digestion, and then down-regulated uptake back to a basal level. In contrast, the two frequent feeders experienced no significant ($P > 0.12$) variation in intestinal nutrient uptake following feeding or on the completion of digestion for any of the three nutrients.

Uptake measurements are available for all eight species at 1 d postfeeding (Fig. 4). At that time, all four frequent feeders exhibited no significant ($P > 0.1$) increase in the uptake of any of the five solutes studied, either by the anterior or the distal regions of the small intestine. In contrast, all four infrequent feeders exhibited significantly ($P < 0.026$) increased uptake rates of almost all measured nutrients within 24 h in both the anterior and distal regions of the small intestine.

Fasting uptake rates, averaged over the four frequent feeders, were 93%–367% higher than values averaged over the four infrequent feeders for each of the five nutrients, both in the anterior and distal small intestine. These differences achieved statistical significance for L-aspartic acid, L-leucine, and L-proline. For those same three nutrients, fasting uptakes of all four infrequent feeders were lower than uptakes of any of the four frequent feeders. Conversely, uptakes 1 d postfeeding averaged over the four infrequent feeders were 9%–143% higher than values averaged over the four frequent feeders for most of the five nutrients in both the anterior and distal small intestine (L-leucine, L-lysine, and D-glucose in both regions, and L-proline in the anterior region), but those differences did not achieve statistical significance.

Thus, infrequent feeders up-regulate intestinal nutrient uptake rates on feeding and then down-regulate them on completion of digestion, but frequent feeders maintain activities of those transporters nearly constant. That up-regulation by in-

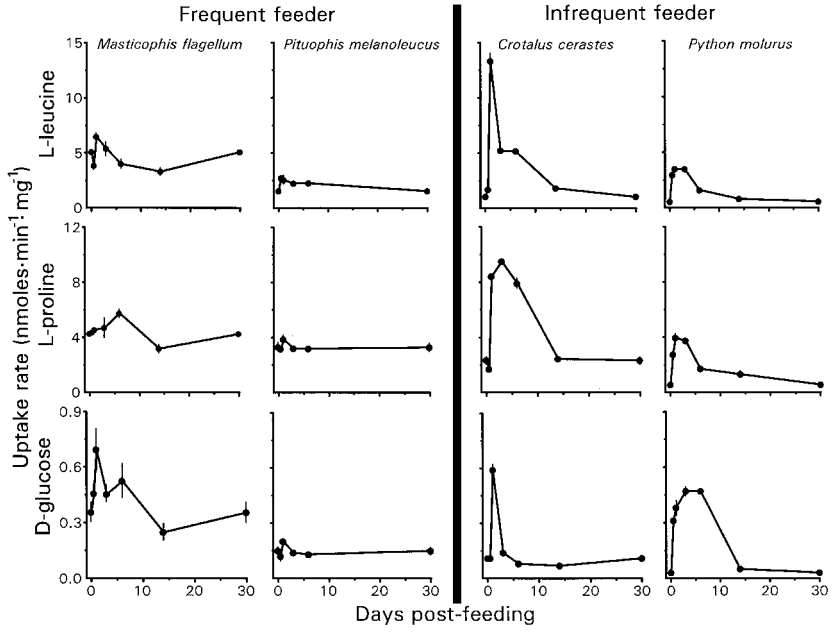



Figure 3. Anterior intestinal brush border uptake rates of the amino acids L-leucine and L-proline and of the sugar D-glucose as a function of time postfeeding for frequently feeding *Masticophis flagellum* and *Pituophis melanoleucus* and for infrequently feeding *Crotalus cerastes* and *Python molurus*. In this figure and in Figures 4–7, $n = 3$ for each sampling period for each species. Note that the two frequent feeders experience no significant changes in intestinal uptake rates on feeding, whereas both infrequent feeders significantly up-regulate the uptake of all three nutrients on feeding and then down-regulate them again on completion of digestion.

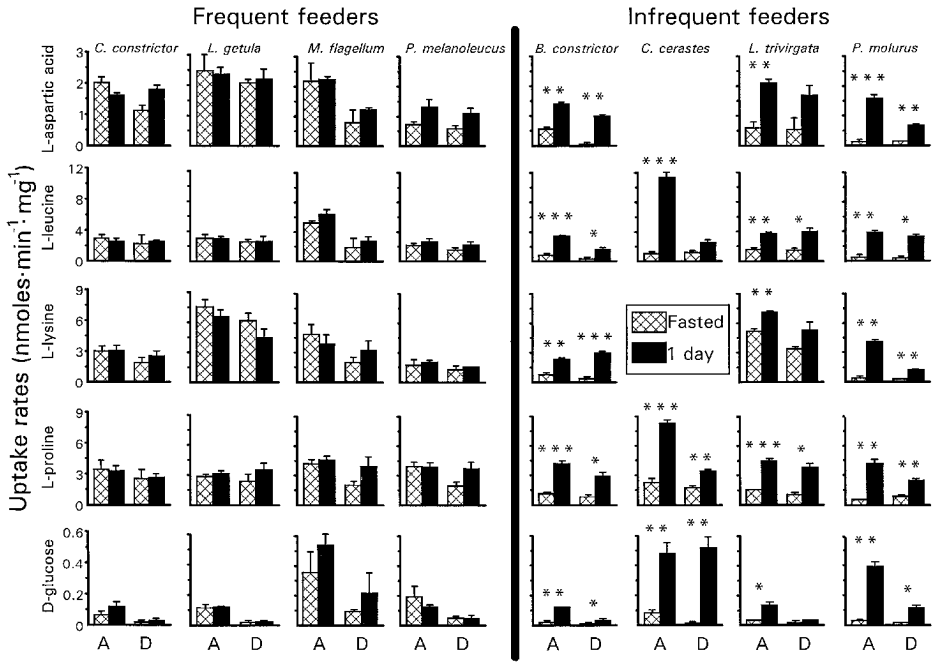



Figure 4. Uptake rates of the amino acids L-aspartic acid, L-leucine, L-lysine, and L-proline and of the sugar D-glucose by the anterior (*A*) and distal (*D*) portions of the small intestine for each of eight snake species after a 30-d fast (fasted, *cross-hatched bars*) and at 1 d postfeeding (1 d, *solid bars*). Note that none of the four frequently feeding snake species experiences a significant increase in the uptake of any measured solute in the anterior or distal intestinal region at 1 d postfeeding, whereas all four infrequently feeding species experience significant increases with feeding for all measured solutes in the anterior region and, in most cases, in the distal region as well. In this and subsequent figures, levels of significance (for change on feeding) are illustrated with asterisks, where one signifies $P < 0.05$, two signify $P < 0.01$, and three signify $P < 0.001$.

frequent feeders especially involves lower fasting uptakes and possibly somewhat higher fed uptakes than for frequent feeders.

### *Organ Masses*

Figure 5 illustrates the postfeeding changes in liver and small intestinal mass for the four species studied at different times after feeding. Both infrequent feeders exhibited significant changes in organ masses with time after feeding: small intestine wet and dry masses and liver and stomach wet masses varied significantly ($P < 0.04$) for both species, while heart, lung, and kidney wet and dry masses varied significantly ($P < 0.03$) for *P. molurus*. The changes consisted of rapid increases of organ masses up to peak values at 1–3 d postfeeding, followed by slower declines back to fasting masses (Fig. 5). In contrast, neither of the frequent feeders exhibited significant variation with time in the wet or dry masses of any weighed organ (Fig. 5).

Organ mass measurements are available for all species at 1 d postfeeding (Tables 2, 3). At that time, all four frequent feeders exhibited no significant change in any of the eight organs measured (except for kidney wet mass in *P. melanoleucus*). In contrast, all four infrequent feeders exhibited significant ($P < 0.046$) increases in wet and dry masses of the small intestine (by 70%–150%) and liver (by 30%–67%), while wet and/or dry masses of the stomach, pancreas, and kidney increased by up to 50%–86% in one to three of these four species.

We also analyzed relative organ masses (i.e., organ mass divided by body mass) in fasting snakes and in snakes at 1 d postfeeding. During fasting, three of the four infrequent feeders possessed significantly ($P < 0.037$) larger stomachs and/or large intestines than all of the four frequent feeders, but the four frequent feeders possessed, on the average, larger (by up to 85%) lungs, hearts, livers, pancreases, small intestines, and kidneys than the four infrequent feeders. Only for lung wet mass ($P = 0.007$) was that larger organ size of the frequent feeders statistically significant, but the differences for pancreas ($P = 0.059$) and heart ($P = 0.081$) wet mass approached significance. Conversely, at 1 d postfeeding, organ masses averaged over the four frequent feeders were generally similar to those averaged over the four infrequent feeders, except that lung mass was 66% higher in the frequent feeders ($P = 0.03$) and large intestine mass was 78% higher in the infrequent feeders ($P = 0.046$).

Thus, frequent feeders exhibited no significant changes in organ masses on feeding, but masses of all measured organs, except the large intestine, increased on feeding in one or more

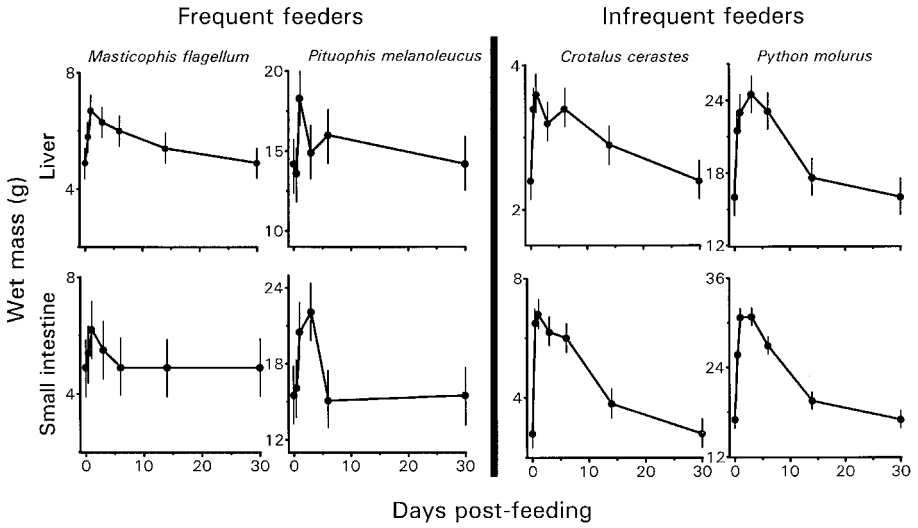



Figure 5. Wet masses of the liver (*top*) and small intestine (*bottom*) of frequently feeding *Masticophis flagellum* and *Pituophis melanoleucus* and infrequently feeding *Crotalus cerastes* and *Python molurus* as a function of time postfeeding. Note that changes with time in the two frequent feeders are not significant ($P > 0.05$ by ANOVA), whereas both infrequent feeders experience significant postfeeding increases in the wet mass of the liver and small intestine ($P < 0.013$), followed by atrophy of both of these organs once digestion has been completed.

of the infrequent feeders. That up-regulation in the infrequent feeders involved smaller organ masses during fasting (except for the stomachs and large intestines) and similar organ masses after feeding when compared with frequent feeders.

### *Intestinal Nutrient Uptake Capacity*

Of the four snake species studied at different time points, both infrequent feeders experienced significant ($P < 0.0005$) changes in intestinal uptake capacities through a feeding bout (Fig. 6). Uptake capacities of all measured nutrients increased steeply by six- to 20-fold within 1 d of feeding, remained elevated during meal digestion, and then declined after digestion was completed. In contrast, uptake capacities of the two frequent feeders did not vary significantly ($P > 0.08$) as a function of time postfeeding (Fig. 6).

At 1 d postfeeding all four frequent feeders exhibited no significant change in uptake capacity of any of the five nutrients, except for an increase ($P = 0.005$) in L-aspartic acid capacity of *P. melanoleucus* (Fig. 7). In contrast, all four infrequent feeders exhibited significant ($P = 0.042$) five- to 30-fold increases in uptake capacities of all five nutrients (Fig. 7).

Just as we did for organ masses, we also analyzed relative uptake capacities (i.e., normalized to snake body mass) in the fasting state and at 1 d postfeeding. Fasting capacities of frequent feeders averaged 2.2- to 9.3-fold higher than those of infrequent feeders for all five nutrients. Conversely, at 1 d postfeeding, the capacities of infrequent feeders averaged 1.6- to 5.4-fold higher than those of frequent feeders for all five nutrients.

Thus, uptake capacities tend to be higher in frequent feeders than infrequent feeders while fasting, because both uptake rates and intestinal mass (whose integrated product equals uptake capacity) are higher in the fasting frequent feeders. The converse is true at 1 d postfeeding: uptake capacities tend to be higher in infrequent feeders than frequent feeders. Only the infrequent feeders undergo large increases in uptake capacity on feeding, as a result of large increases in both uptake rates and intestinal mass.

### *Phylogenetically Independent Contrast Analysis*

Because all four of our frequently feeding snake species belong to the family Colubridae, whereas our four infrequent feeders are divided among three other families, one might question whether the species differences that we have been describing might just be phylogenetically linked characters of colubrid snakes, unrelated to feeding frequency. To test this possibility, Figure 8 compares six sets of data: our reconstructed phylogeny of the eight species, each species' estimated feeding interval as adults in the wild, its SDA, and its factorial 1-d-postfeeding increases in L-leucine, L-proline, and D-glucose intestinal uptake capacities. The eight species yield seven standardized contrasts, as explained in the legend to Figure 9. We found positive and significant ($t$ values = 2.97–3.92, $P < 0.02$) correlations between phylogenetically independent contrasts in feeding inter-

Table 2: Body masses and wet and dry masses (least square means ± 1 SE) of organs removed from fasted and fed (1 d postfeeding) frequently feeding snake species

| | *Coluber constrictor* | | *Lampropeltis getula* | | *Masticophis flagellum* | | *Pituophis melanoleucus* | |
|---|---|---|---|---|---|---|---|---|
| | Fasted | 1 d pf | Fasted | 1 d pf | Fasted | 1 d pf | Fasted | 1 d pf |
| Body mass (g) | 172 ± 28 | 167 ± 27 | 223 ± 23 | 216 ± 39 | 310 ± 134 | 348 ± 90 | 760 ± 339 | 723 ± 165 |
| Wet mass: | | | | | | | | |
| Heart | .9 ± .1 | .7 ± .1 | .7 ± .1 | .7 ± .1 | 1.7 ± .1 | 1.4 ± .1 | 3.3 ± .2 | 3.2 ± .2 |
| Lung | 2.4 ± .2 | 1.6 ± .2 | 3.6 ± .3 | 3.7 ± .3 | 3.3 ± .3 | 3.7 ± .3 | 8.7 ± .6 | 7.8 ± .6 |
| Liver | 3.6 ± .4 | 3.9 ± .4 | 5.7 ± .2 | 6.4 ± .2 | 5.2 ± .4 | 7.1 ± .4 | 15.1 ± 1.9 | 18.4 ± 1.9 |
| Stomach | 3.2 ± .3 | 4.3 ± .3 | 4.4 ± .6 | 7.2 ± .6 | 6.0 ± 1.3 | 6.5 ± 1.3 | 12.3 ± 1.2 | 14.9 ± 1.2 |
| Pancreas | .3 ± .1 | .4 ± .1 | .5 ± .1 | .6 ± .1 | .5 ± .1 | .5 ± .1 | 1.3 ± .2 | 1.5 ± .2 |
| Small intestine | 4.1 ± .4 | 5.6 ± .4 | 4.6 ± .5 | 6.9 ± .5 | 5.1 ± .5 | 6.4 ± .5 | 12.1 ± 2.4 | 20.4 ± 2.4 |
| Large intestine | 1.4 ± .2 | 1.5 ± .2 | 1.5 ± .1 | 1.6 ± .1 | 2.4 ± .2 | 2.9 ± .2 | 4.6 ± .6 | 5.2 ± .6 |
| Kidney | 1.6 ± .3 | 1.5 ± .3 | 1.4 ± .1 | 1.8 ± .1 | 2.0 ± .2 | 1.8 ± .2 | 3.6 ± .1 | 4.3 ± .1* |
| Dry mass: | | | | | | | | |
| Heart | .17 ± .01 | .14 ± .01 | .13 ± .01 | .14 ± .01 | .26 ± .02 | .27 ± .02 | .59 ± .07 | .58 ± .07 |
| Lung | .5 ± .1 | .3 ± .1 | .6 ± .1 | .7 ± .1 | .6 ± .1 | .8 ± .1 | 1.5 ± .2 | 1.5 ± .2 |
| Liver | .8 ± .1 | .9 ± .1 | 1.6 ± .1 | 1.5 ± .1 | 1.4 ± .2 | 1.8 ± .2 | 4.2 ± .5 | 4.7 ± .5 |
| Stomach | .6 ± .1 | .8 ± .1 | .9 ± .1 | 1.2 ± .1 | 1.2 ± .3 | 1.2 ± .3 | 2.5 ± .2 | 3.1 ± .2 |
| Pancreas | .06 ± .01 | .09 ± .01 | .14 ± .02 | .14 ± .02 | .11 ± .01 | .10 ± .01 | .31 ± .04 | .35 ± .04 |
| Small intestine | .6 ± .1 | 1.0 ± .1 | .8 ± .1 | 1.3 ± .1 | .8 ± .1 | 1.0 ± .1 | 2.1 ± .4 | 3.2 ± .4 |
| Large intestine | .25 ± .04 | .21 ± .04 | .24 ± .02 | .29 ± .02 | .50 ± .05 | .54 ± .05 | .7 ± .1 | .7 ± .1 |
| Kidney | .32 ± .08 | .29 ± .08 | .29 ± .02 | .32 ± .02 | .45 ± .06 | .38 ± .06 | .8 ± .1 | .9 ± .1 |

Note. Organs were weighed from three fasted and three fed individuals of each species. Differences in organ mass between fasted and fed individuals were tested by ANCOVA. pf = postfeeding.
* $0.05 > P \geq 0.01$.

Table 3: Body masses and wet and dry masses (least square means ± 1 SE) of organs removed from fasted and fed (1 d postfeeding) infrequently feeding snake species

| | *Boa constrictor* | | *Crotalus cerastes* | | *Lichanura trivirgata* | | *Python molurus* | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Fasted | 1 d pf | Fasted | 1 d pf | Fasted | 1 d pf | Fasted | 1 d pf |
| Body mass (g) | 366 ± 75 | 383 ± 87 | 132 ± 10 | 125 ± 6 | 221 ± 49 | 250 ± 63 | 703 ± 139 | 746 ± 73 |
| Wet mass: | | | | | | | | |
| Heart | 1.2 ± .1 | 1.6 ± .1 | .5 ± .1 | .6 ± .1 | .6 ± .1 | .7 ± .1 | 2.8 ± 1.1 | 3.9 ± 1.1 |
| Lung | 2.7 ± .3 | 2.4 ± .3 | 1.2 ± .1 | 1.1 ± .1 | 1.5 ± .1 | 1.7 ± .1 | 4.0 ± .5 | 4.4 ± .5 |
| Liver | 6.2 ± .3 | 9.1 ± .3** | 2.6 ± .3 | 3.8 ± .3* | 5.4 ± .2 | 7.5 ± .2** | 15.9 ± 1.5 | 22.9 ± 1.5* |
| Stomach | 8.2 ± .5 | 8.6 ± .5 | 3.0 ± .1 | 3.4 ± .1** | 3.6 ± .4 | 6.2 ± .4* | 14.4 ± .9 | 19.0 ± .9* |
| Pancreas | .53 ± .01 | .92 ± .01*** | .10 ± .02 | .16 ± .02 | .32 ± .02 | .36 ± .02 | .9 ± .2 | 1.3 ± .2 |
| Small intestine | 8.8 ± .9 | 18.1 ± .9** | 1.4 ± .2 | 3.5 ± .2** | 3.3 ± .7 | 8.3 ± .7* | 16.3 ± 2.3 | 30.6 ± 2.3* |
| Large intestine | 7.9 ± .3 | 7.2 ± .3 | 1.0 ± .1 | 1.5 ± .1 | 1.8 ± .2 | 2.3 ± .2 | 12.8 ± .7 | 13.4 ± .7 |
| Kidney | 2.1 ± .2 | 3.1 ± .2* | .7 ± .1 | 1.0 ± .1 | 1.3 ± .1 | 2.0 ± .1** | 3.5 ± .5 | 6.5 ± .5* |
| Dry mass: | | | | | | | | |
| Heart | .21 ± .03 | .24 ± .03 | .10 ± .02 | .11 ± .02 | .12 ± .01 | .13 ± .01 | .5 ± .2 | .6 ± .2 |
| Lung | .41 ± .01 | .42 ± .01 | .20 ± .01 | .20 ± .01 | .26 ± .02 | .30 ± .02 | .8 ± .1 | .9 ± .1 |
| Liver | 2.0 ± .2 | 2.7 ± .2* | .6 ± .1 | 1.0 ± .1* | 1.6 ± .1 | 2.2 ± .1* | 5.6 ± .5 | 7.22 ± .5* |
| Stomach | 1.5 ± .1 | 1.6 ± .1 | .6 ± .1 | .6 ± .1 | .7 ± .1 | 1.0 ± .1* | 2.7 ± .3 | 3.4 ± .3* |
| Pancreas | .11 ± .02 | .17 ± .02 | .02 ± .01 | .04 ± .01 | .07 ± .01 | .07 ± .01 | .19 ± .02 | .26 ± .02 |
| Small intestine | 1.4 ± .2 | 3.3 ± .2** | .3 ± .1 | .6 ± .1* | .6 ± .1 | 1.4 ± .1** | 3.1 ± .4 | 5.2 ± .4* |
| Large intestine | 1.0 ± .1 | 1.0 ± .1 | .18 ± .02 | .24 ± .02 | .24 ± .03 | .32 ± .03 | 2.1 ± .2 | 2.0 ± .2 |
| Kidney | .38 ± .02 | .52 ± .02* | .17 ± .02 | .18 ± .02 | .26 ± .01 | .33 ± .01* | .8 ± .1 | 1.2 ± .1* |

Note. Organs were weighed from three fasted and three fed individuals of each species. Differences in organ mass between fasted and fed individuals were tested by ANCOVA. pf = postfeeding.

* $0.05 > P \geq 0.01$.

** $0.01 > P \geq 0.001$.

*** $P < 0.001$.

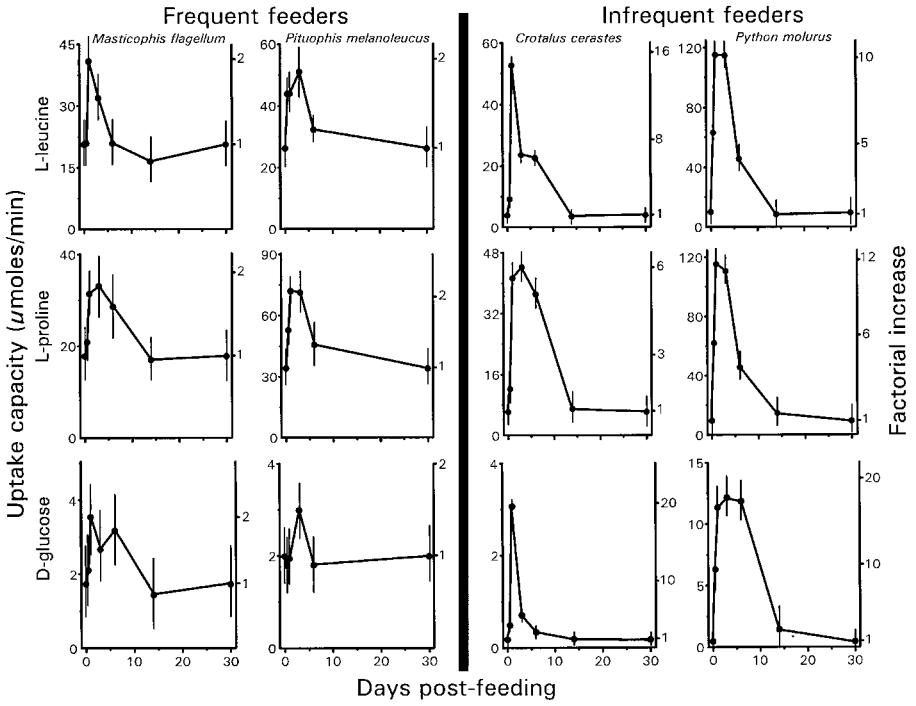



Figure 6. Uptake capacities of the whole length of the small intestine for the amino acids L-leucine and L-proline and the sugar D-glucose as a function of time postfeeding for frequently feeding *Masticophis flagellum* and *Pituophis melanoleucus* and infrequently feeding *Crotalus cerastes* and *Python molurus*. Note that the two frequently feeding snake species experience only a modest twofold change (not statistically significant) in intestinal uptake capacities, whereas the two infrequently feeding species experience reversible seven- to 24-fold increases (significant in all cases).

vals and the contrasts in SDA ($r = 0.77$), in factorial scope of peak $\dot{V}O_2$ ($r = 0.85$, not depicted in Fig. 9), and in factorial capacity increases for L-leucine ($r = 0.81$), L-proline ($r = 0.83$), and D-glucose ($r = 0.85$). That is, even after phylogeny has been taken into account, species with long feeding intervals still experience a higher cost of digestion (SDA), higher factorial scope of peak $\dot{V}O_2$, and higher postfeeding up-regulation of intestinal uptake capacities than do species with short feeding intervals.

## Discussion

We shall discuss, in turn, the combination of large feeding responses with small fasting capacities in infrequently feeding snakes; correlations of low SMR in infrequent feeders; costs and benefits of regulation, including a quantitative model that may explain the evolution of large feeding responses; and proposed further tests of our hypothesis among snakes and among taxa other than snakes.

### *Feeding Responses and Fasting Capacities*

We undertook this project to test our hypothesis that regulatory scopes of digestion are related to natural feeding habits; that is, that species feeding frequently on small meals experience only modest fluctuations in loads on the gut and require only modest regulatory responses, whereas species feeding infrequently on large meals evolve larger regulatory responses.

Our comparison of four frequently feeding and four infrequently feeding snake species support this hypothesis. For the four infrequent feeders, feeding induced within a day 10- to 17-fold increases in aerobic metabolism, 90%–180% increase in small intestinal mass, 37%–98% increases in masses of other organs active in nutrient processing, three- to 16-fold increases in intestinal nutrient transport rates, and five- to 30-fold increases in intestinal nutrient uptake capacities. As demonstrated for *Crotalus cerastes* and *Python molurus*, the two infrequent feeders that we studied at many time points, we were able to trace how all of these responses reverted to fasting levels on the completion of digestion. In contrast, when we administered the same relative meal size (equivalent to 25% of snake body mass) to the four frequent feeders, there were virtually no significant changes in masses of the small intestine and other organs, nutrient transport rates, or nutrient uptake capacities. The frequent feeders exhibited more modest increases in aerobic metabolism (by only five- to eightfold) than the infrequent feeders and completed digestion more rapidly.

Do those larger responses of infrequent feeders to feeding

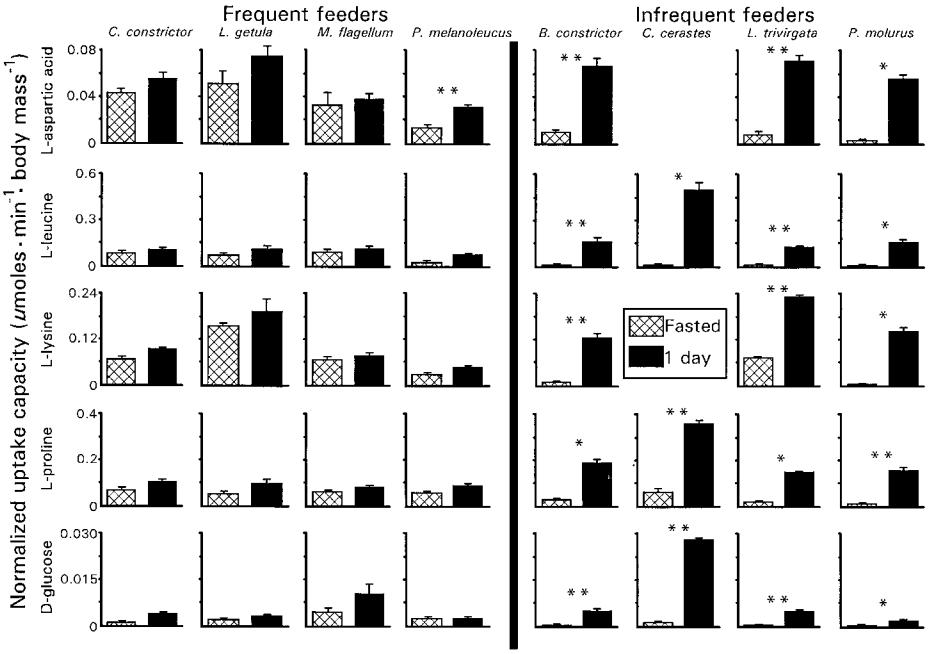



Figure 7. Total intestinal uptake capacities normalized to body mass for the amino acids L-aspartic acid, L-leucine, L-lysine, and L-proline and the sugar D-glucose, in eight snake species when fasted and also at 1 d postfeeding. Note that all four frequently feeding species experience no significant change in solute uptake capacity (with the exception of L-aspartic acid for *Pituophis melanoleucus*) after feeding, whereas all four infrequently feeding snakes experience large (five- to 30-fold) and significant ($P \leq 0.042$) increases in capacity for all measured nutrients.

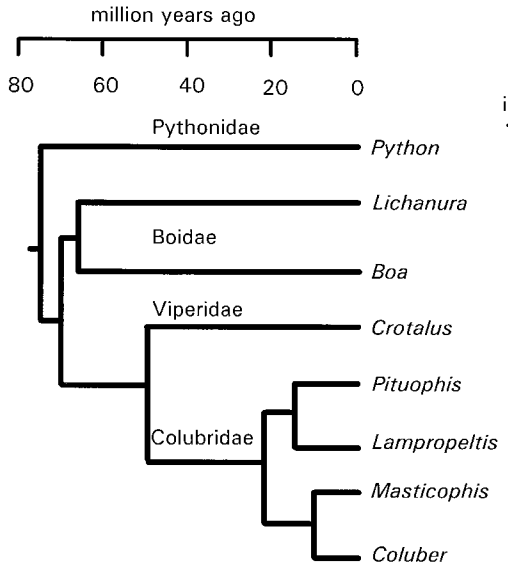
result from lower fasting levels (of $\dot{V}O_2$, organ masses, and transport rates and capacities), higher fed levels, or both? It turns out that lower fasting levels contribute to all responses and higher fed levels to some responses. Averaged over the four infrequent feeders, fasting SMR is 55% lower, uptake rates 48%–79% lower, organ masses up to 46% lower, and uptake capacities 54%–89% lower than for the four frequent feeders. In contrast, fed uptake rates are 9%–143% and uptake capacities are 64%–439% higher for infrequent feeders than for frequent feeders; peak $\dot{V}O_2$ is 58% higher for two of the infrequent feeders, but similar to the frequent feeders for the other two infrequent feeders; and fed organ masses of infrequent feeders are higher for the small intestine and large intestine but similar to the frequent feeders for other organs.

### *Correlates of Low SMR in Infrequent Feeders*

Might the low fasting levels of nutrient transporters and organ masses contribute to the observed reduction of fasting metabolic rate (SMR) in infrequent feeders? To test for a possible association between SMR and transporter activities or organ masses independent of body mass, we regressed for all eight species of fasted snakes their SMR, intestinal uptake capacities for L-leucine and L-proline and D-glucose, and wet masses of all eight weighed organs against body mass. We then tested for correlations between the residuals from the regressions of intestinal uptake capacities or organ masses versus body mass against the residuals from the regression of SMR versus body mass.

As illustrated in Figure 10, the two sets of residuals were indeed correlated, with residuals for infrequent feeders located mostly in the lower left quadrants (both SMR and uptake capacity or organ mass lower than expected for body mass) and with the residuals for frequent feeders located mostly in the upper right quadrants (both SMR and uptake capacity or organ mass higher than expected for body mass). Correlations against residuals of SMR were positive and significant ($r = 0.47$–$0.76$, $P = 0.0001$–$0.02$) for residuals of all three measured uptake capacities (L-leucine, L-proline, D-glucose) and of five of the eight measured organs (heart, lungs, pancreas, small intestine, kidneys). The same conclusion emerges if, instead of analyzing values for each individual snake (as we do in Figure 10, total $N = 24$), we analyze the average residual values for each snake species ($N = 8$). That is, among these eight snake species, species with high or low SMR tend to have respectively high or low uptake capacities and organ masses.

Two organs, the stomach and large intestine, deviate from the pattern of Figure 10 and from previously discussed trends in organ mass. That is, both of these organs proved to be larger in fasted infrequent feeders than in frequent feeders—the re-




| | Feeding interval (d) | SDA (kJ/kg) | Factorial increase in uptake capacity of: L-leucine | L-proline | D-glucose |
|---|---|---|---|---|---|
| *Python* | 40 | 580 | 12 | 12 | 29 |
| *Lichanura* | 30 | 360 | 4.6 | 6.0 | 8.3 |
| *Boa* | 35 | 670 | 9.7 | 7.2 | 9.4 |
| *Crotalus* | 38 | 460 | 13 | 5.0 | 17 |
| *Pituophis* | 14 | 290 | 1.5 | 1.8 | 1.9 |
| *Lampropeltis* | 12 | 300 | 2.6 | 2.4 | 2.2 |
| *Masticophis* | 10 | 260 | 2.2 | 2.0 | 2.3 |
| *Coluber* | 8 | 310 | 1.4 | 1.6 | 1.9 |

Figure 8. *Left*, Reconstructed phylogenetic relationships and estimated divergence times (millions of years) for the eight snake species used in this study. *Right*, Variables used in our phylogenetically independent contrast analyses. Feeding interval is the approximate duration (*d*) between meals for adult individuals in the wild. SDA (specific dynamic action) is the calculated energetic cost (kJ $kg^{-1}$) of digestion. Factorial increases in nutrient uptake capacities were calculated by dividing mean values for fed snakes (1 d postfeeding) by mean values for fasted snakes.

verse of the patterns for other organs. Figure 10 suggests, and we shall argue further in the next section, that infrequent feeders can save energy while fasting by reducing masses of most energetically expensive organs and by up-regulating these organ masses only after the snake has eaten. How can these two exceptions be explained?

For the stomach the answer is obvious: infrequent feeders consume, on average, larger prey than frequent feeders. Hence, infrequent feeders require a large stomach immediately on ingestion of the prey and cannot wait a day to grow a large stomach. The other exception involves the large intestine: snakes of the families Boidae and Pythonidae (which include three of our four infrequent feeders but not our five snakes of other families) possess a cecal-like extension of the large intestine that contributed to the larger mass reported for the large intestine in these three species.

### *Costs and Benefits of Regulation*

Why would infrequently feeding snakes have evolved to down-regulate the intestine and other organs after completing digestion, given the high cost of up-regulation on feeding? And why would frequently feeding snakes have evolved not to regulate those organs up and down throughout their feeding cycle?

One likely driving force behind down-regulation is energy savings during fasting, that is, reducing SMR. For ambush-hunting snakes, whose annual costs of activity are low, SMR contributes a large fraction of the annual energy budget (e.g., 37% for *C. cerastes*; Secor and Nagy 1994). SMRs of our four infrequent feeders proved to average 55% less than SMR of our frequent feeders. That translates into a 15% reduction in the infrequent feeders' annual energy budget (data from Secor and Nagy 1994), energy that could potentially be devoted to growth, fat storage, and/or reproduction.

SMR may be considered as the sum of metabolic rates of individual organs and tissues (Schmidt-Nielsen 1984). Organs with high mass-specific metabolic rates that contribute disproportionately to SMR include the kidneys, small intestine, heart, and liver (Field et al. 1939). All four of those organs, plus some others, are ones on which infrequently feeding snakes place reduced or even no functional demands while fasting. The most obvious candidates for down-regulation are the small intestine and pancreas, which perform essentially no work during fasts, and liver and kidneys, because of lower metabolic turnover and waste production during fasts. There is also much less work for the heart and lungs (significant decline in heart and respiration rates during fasts for *P. molurus*; S. M. Secor, J. W. Hicks, and A. F. Bennett, unpublished observations); this may be reflected in the significant decline in mass of these two organs after completion of digestion in *P. molurus* (Secor and Diamond 1995). Hence, fasting snakes can save energy by letting these organs atrophy and can save further energy by down-regulating specific transporters and enzymes, as observed for intestinal nutrient transporters (this article) and intestinal hydrolases (Secor and Diamond 1998). Our data yield two expressions of these energy considerations: (1) the correlations among all eight

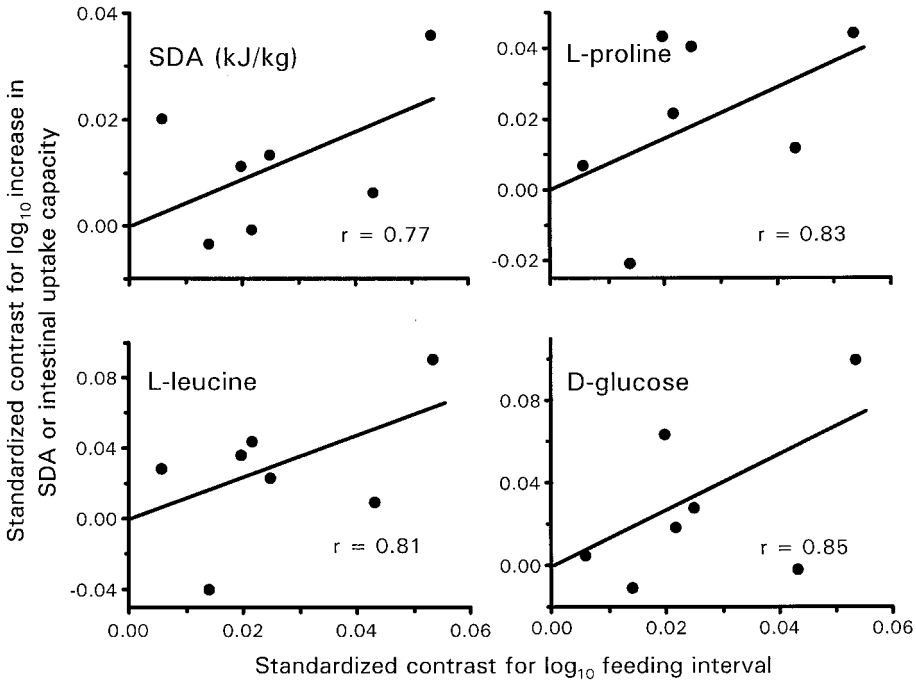



Figure 9. Bivariate plots of standardized contrasts for SDA or $\log_{10}$ factorial increase in intestinal nutrient uptake capacity (L-leucine, L-proline, and D-glucose) versus standardized contrasts for $\log_{10}$ feeding interval. Contrasts were calculated by methods of phylogenetically independent contrasts (Felsenstein 1985) that integrate the topology of the phylogeny with each set of parameter values presented in Figure 8. Before analysis, contrasts were standardized by the square root of summed branch lengths. In each plot the regression line is forced through the origin, and each correlation coefficient is significant at $P < 0.02$.

fasting snake species between SMR and organ masses or intestinal uptake; and (2) lower SMRs, organ masses, and nutrient uptake rates of infrequent feeders compared with frequent feeders while fasting.

Offsetting the energy saved by reducing organ masses and transporter activities (thereby reducing SMR) during fasting are the costs of up-regulation (i.e., of rebuilding these organs and transporters) on feeding. We suggest that the reason why infrequent feeders down-regulate on completing digestion is that the resulting energy savings during their long fasts offset the occasional high costs of up-regulation, while the reason why frequent feeders do not down-regulate is that they would thereby incur too often (with each meal) the high costs of up-regulation.

We tested this hypothesis by constructing a simple model that compares the time-averaged partial daily energy budget as a function of intermeal interval for one frequently and one infrequently feeding snake species. Using actual data from field and laboratory studies on the frequently feeding *Masticophis flagellum* and the infrequently feeding *C. cerastes* (Secor and Nagy 1994; Secor et al. 1994; this article), we calculated, for similar-sized snakes of each species as a function of feeding interval ranging from 1 to 8 wk, the time-averaged daily energy expenditure resulting from the sum of two components SMR (fasting metabolism) plus SDA (costs of up-regulation and digestion). The calculated energy budgets of the two species cross over at a feeding interval of approximately 4 wk (Fig. 11). This suggests that at feeding intervals of <4 wk, a snake with the physiology of the frequent feeder *M. flagellum* has the advantage of the lower energy budget because its higher fasting metabolism (due to maintaining the gut and other organs constantly ready) is more than offset by not having to incur the high cost of up-regulating the gut and other organs at every one of its frequent meals. For instance, at a feeding interval of 1.5 wk, the calculated daily energy budget (SMR plus SDA) of the frequent feeder *M. flagellum* would be 30% lower than that of *C. cerastes*. Conversely, at feeding intervals >4 wk, it is instead a snake with the physiology of the infrequent feeder *C. cerastes* that has the advantage of the lower energy budget because its lower metabolism during the long fasts now more than offsets its occasional high costs of up-regulation. For instance, at a feeding interval of 8 wk, the calculated energy budget of *C. cerastes* would be 20% lower than that of *M. flagellum*. The implications of this model agree with field observations of feeding intervals: *M. flagellum* actually feeds at an average interval of only 10 d, and *C. cerastes* at an interval of 38 d (Secor and Nagy 1994). Hence, energy considerations may explain why

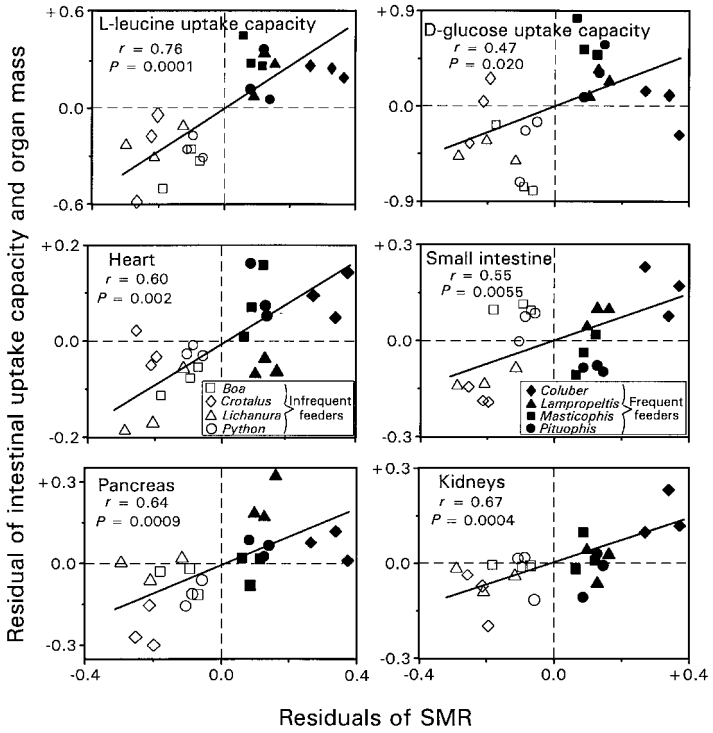



Figure 10. Residual variation in intestinal nutrient uptake capacity or organ mass (ordinate) plotted against residual variation in SMR (abscissa) for the pooled sample of all eight species of snakes. Residuals were taken from regressions of uptake capacity, organ mass, or SMR against body mass of fasted snakes. Note that residual values for the infrequently feeding snakes or frequently feeding snakes are clustered in the lower left or upper right quadrant, respectively. That is, snakes with below-average uptake capacities and organ masses during fasting tend to possess below-average SMR and to be infrequent feeders, whereas snakes with above-average uptake capacities and organ masses during fasting tend to possess above-average SMR and to be frequent feeders.

infrequent feeders regulate organ performance and size while frequent feeders do not.

Another hypothesis, which is not mutually exclusive of this energy hypothesis, is that the occurrence or nonoccurrence of regulation is driven by foraging technique. Our four frequent feeders are also active foragers, so that their large hearts, lungs, and kidneys during the fasting state (on the average, respectively, 31%, 85%, and 28% larger than those of the infrequent feeders) might be required to support their more athletic lifestyles—whereas three of our four infrequent feeders are sit-and-wait foragers that are less active while fasting. However, this hypothesis does not explain why the actively foraging frequent feeders also have large pancreases and small intestines while fasting, nor why the infrequent feeder *Lichanura trivirgata* (which often forages actively, like our four frequent feeders) down-regulates its organs while fasting, like the other infrequent feeders.

### *Further Tests of Our Hypothesis*

We suggest two extensions of this project to further studies on snakes: incorporation of an improved phylogeny and measurements on additional snake species. First, because a rigorous phylogeny of snakes that includes our eight study species was not available, we pieced together our phylogeny (Fig. 8) from available morphological, immunological, and molecular information. Our sources for natural feeding intervals ranged from anecdotal field observations to detailed ecological studies. We did test the robustness of our conclusions generated by the phylogenetic analysis in four ways: (1) by setting the various branch lengths of the phylogeny of Figure 8 equal to each other; (2) by increasing the feeding intervals of frequent feeders and decreasing the intervals of infrequent feeders (or vice versa) by 25%; (3) by removing each one of the eight species in turn from the analysis; and (4) by condensing the data to the family level (Pythonidae, Boidae, Viperidae, and Colubridae). None of these procedures altered the statistical significance of our conclusions. Nevertheless, an obvious future research program will be to obtain extensive field data on feeding intervals and to construct a rigorous phylogeny with firmly established ancestral nodes and branch lengths.

Second, considerations of snake evolutionary history (Greene 1997) suggest that the large regulatory responses we observed

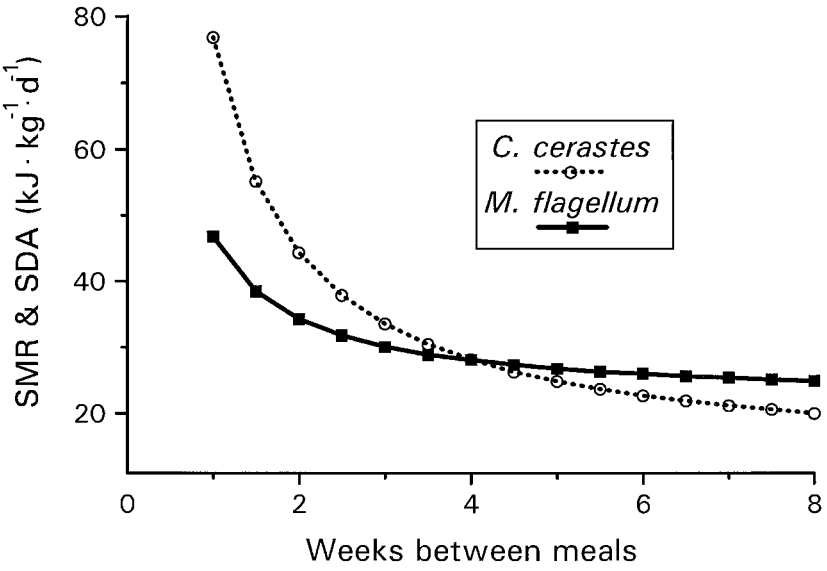



Figure 11. Time-averaged partial daily energy expenditure calculated as the sum of standard metabolic rate (SMR) and specific dynamic action (SDA) as a function of feeding interval (wk) for free-ranging infrequently feeding *Crotalus cerastes* and frequently feeding *Masticophis flagellum*. For each feeding interval (1–8 wk), average daily SMR and SDA were calculated by integrating field measurements of body temperature, field measurements of relative meal sizes (15% and 25% of body mass for *M. flagellum* and *C. cerastes*, respectively), and laboratory measurements of SMR and SDA (Secor and Nagy 1994; Secor et al. 1994; Secor 1995*a*; S. M. Secor, unpublished observations). For example, at a feeding interval of 2 wk, the daily expenditure of 44.4 kJ $kg^{-1}$ $d^{-1}$ for *C. cerastes* consists of the average energy expended daily on digesting a meal 25% of body mass (SDA = 455 kJ $kg^{-1}$ [Table 1] divided by 14 d) plus SMR (11.9 kJ $kg^{-1}$ $d^{-1}$). Note that, at feeding intervals of <4 wk, *C. cerastes* is projected to have higher time-averaged energy expenditure than *M. flagellum* (due to frequently incurring its high cost of digestion), whereas at intervals >4 wk, *C. cerastes* has a lower time-averaged energy expenditure because of *M. flagellum*'s much higher fasting energy expenditure (field SMR 85% greater than that of *C. cerastes* when field differences in body temperature are taken into account [Secor and Nagy 1994]).

for four snake species may have evolved, been lost, and reevolved repeatedly during snake history. The earliest snakes are thought to have been small and to have fed on small arthropods, as is still true of the more primitive of the two basal branches of living snakes (Scolecophidia, blind snakes; see Fig. 2 of Greene 1997). The other basal branch, Alethinophidia, comprising all other living snakes, evolved innovations in skull morphology leading to a larger gape plus the ability to constrict prey. These two innovations may have permitted early Alethophidian snakes to take large prey items and to feed infrequently, as is true of the relatively primitive Alethophidian snake families Boidae and Pythonidae today. The Miocene's explosive radiation of snakes saw the rise to dominance of a new superfamily, the Colubroidea, of which one family (Viperidae) is still dominated by sit-and-wait-foraging infrequent feeders, while most species in the other three families (Atractaspididae, Elapidae, and Colubridae) are actively foraging frequent feeders. But within the Elapidae, one species, the Australian death adder (*Acanthophis*), is a sit-and-wait-foraging infrequent feeder (Shine 1980), and within the Colubridae, the African egg-eating snakes (*Dasypeltis*) feed solely on birds' eggs, a resource whose predictable seasonality may require the snakes to fast for several months of the year.

If large regulatory responses to feeding are indeed associated with infrequent consumption of large meals, that would suggest that those responses evolved in early Alethinophidian snakes, were then lost in colubroid snakes other than the family Viperidae, and reevolved thereafter in *Acanthophis* and *Dasypeltis*. Hence we predict that large responses will be found in infrequently feeding species of Viperidae, *Acanthophis*, and *Dasypeltis* and will not be found in frequently feeding species of Atractaspididae and Elapidae.

Our hypothesis about an association between natural feeding interval and regulation of gut performance also lends itself to testing in other animals besides snakes, especially in ectotherm groups that include species with limited energy budgets and long episodes of digestive quiescence. We have found that 11 frequently feeding species of frogs (*Hyla*, *Rana*), salamanders (*Taricha*), turtles (*Chelydra*, *Sternotherus*, *Trachemys*), and lizards (*Gambelia*, *Sceloporus*) exhibit only very small postfeeding responses of gut and metabolic physiology, comparable with those of our four frequently feeding snake species (Secor and

Diamond 1996, 1999; S. M. Secor and J. M. Diamond, unpublished observations). On the other hand, two frog species (the South American horned frog *Ceratophrys ornata* and the African bullfrog *Pyxicephalus adspersus*) and one lizard species (the African white-throated savannah monitor *Varanus albigularis*) that fast seasonally for many months in the wild exhibit large postfeeding responses in metabolic rate and intestinal nutrient capacity, comparable with those of our infrequently feeding snakes (Secor and Diamond 1996; Secor and Phillips 1997). Many other amphibian and reptile species provide contrasts in natural feeding habits, as do fish. These and other species can be used to test the generality of our hypothesis.

## Acknowledgments

We thank D. Do, I. Chadnary, C. Entwisle, M. Esteverena, J. Isaacs, J. Johns, K. Kim, D. Lee, L. Pham, R. Schroeder, K. Searcy, K. Shiotani, C. Slotnick, J. Smith, S. Szabo, R. Torres, F. Wayland, and M. Weldon for their assistance with experiments; T. Garland for assistance with the phylogenetic analyses; and A. Bennett for stimulating discussion. Bruce Toloksi of California Fish and Game kindly provided us with *Lampropeltis getula* and *Lanchanura trivirgata*. This work was supported by National Institutes of Health Research Service award DK-08878 and by National Institutes of Health grant GM-14772.

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