

Adaptive regulation of digestive performance in the genus *Python*

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Summary

The adaptive interplay between feeding habits and digestive physiology is demonstrated by the Burmese python, which in response to feeding infrequently has evolved the capacity to widely regulate gastrointestinal performance with feeding and fasting. To explore the generality of this physiological trait among pythons, we compared the postprandial responses of metabolism and both intestinal morphology and function among five members of the genus *Python*: *P. brongersmai*, *P. molurus*, *P. regius*, *P. reticulatus* and *P. sebae*. These infrequently feeding pythons inhabit Africa, southeast Asia and Indonesia and vary in body shape from short and stout (*P. brongersmai*) to long and slender (*P. reticulatus*). Following the consumption of rodent meals equaling 25% of snake body mass, metabolic rates of pythons peaked at 1.5 days at levels 9.9- to 14.5-fold of standard metabolic rates before returning to prefeeding rates by day 6–8. Specific

dynamic action of these meals (317–347 kJ) did not differ among species and equaled 23–27% of the ingested energy. For each species, feeding triggered significant upregulation of intestinal nutrient transport and aminopeptidase-N activity. Concurrently, intestinal mass doubled on average for the five species, in part due to an 85% increase in mucosal thickness, itself a product of 27–59% increases in enterocyte volume. The integrative response of intestinal functional upregulation and tissue hypertrophy enables each of these five python species, regardless of body shape, to modulate intestinal performance to meet the demands of their large infrequent meals.

Key words: Adaptive response, digestion, intestinal enzyme, intestinal nutrient transport, *Python*, reptile, specific dynamic action.

Introduction

The integration of a species' ecology and physiology is exemplified in the adaptive interplay between their feeding ecology and digestive physiology (Karasov and Diamond, 1988; McWilliams et al., 1997; Secor, 2005a). A well-known example of this is the adaptive correlation between food habits (i.e. herbivory and carnivory) and the morphology and function of the gastrointestinal (GI) tract. The GI tract of herbivores is typically longer than that of carnivores and possesses specialized regions for the fermentation of plant material (Stevens and Hume, 1995; Karasov and Hume, 1997; Mackie, 2002). Equally apparent is how organisms are able to face routine fluctuations in the amount and type of food consumed due to seasonal changes in food availability, ontogenetic shifts in diet, reproductive status and alternating foraging and feeding strategies (O'Brien et al., 1989; Stergiou and Fourtouni, 1991; Koertner and Heldmaier, 1995; Johnson et al., 2001). In response to such shifts in feeding habits, and thus digestive demand, species modulate gut performance to match pace with digestive load (Hammond and Diamond, 1994; Piersma and Lindström, 1997; Weiss et al., 1998). The plasticity of GI performance is manifested in morphological restructuring

and/or functional regulation at the cellular level (Ferraris, 1994; Carey, 1990; Secor, 2005a).

The adaptive capacity to regulate digestive performance in response to changes in digestive demand is well expressed by amphibian and reptile species that naturally experience long episodes of fasting (Secor, 2005a). Anurans that estivate during dry seasons and snakes that employ the sit-and-wait tactic of foraging, and thus eat infrequently, severely downregulate GI performance upon the completion of digestion, maintain a quiescent gut while fasting, and with feeding, rapidly upregulate digestive performance (Secor and Diamond, 2000; Secor, 2005b). The benefit of this trait is observed as a reduction in energy expenditure during the bouts of fasting. For example, during estivation, the metabolic rates of anurans are depressed by 70%, and the standard metabolic rates (SMR) of sit-and-wait foraging snakes are 47% less than that of active foraging snakes that only modestly regulate GI performance with feeding and fasting (Guppy and Withers, 1999; Secor and Diamond, 2000).

For sit-and-wait foraging snakes, the correlation between infrequent feeding and wide regulation of intestinal performance has been investigated for only four species

representing three lineages [Boidae, Pythonidae and Viperidae (Secor and Diamond, 2000)]. Given that these lineages are dominated by species that employ the sit-and-wait tactic of foraging, and presumably eat infrequently, it could be hypothesized that the wide regulation of digestive performance is a conserved trait, basal for each of these lineages and expressed by all members. Alternatively, given the species diversity within these lineages, the capacity to modulate gut performance may be linked to species differences in geography, morphology, habitat and feeding ecology. Hence, a recurring question in our research on the adaptive response of the digestive system is whether physiological responses to feeding and fasting are equivalent among sit-and-wait foraging snakes, or if the magnitude of response varies as a function of differences in geography, morphology and/or ecology.

To address this question, we started with a comparative study on the physiological responses to feeding within the genus *Python* (Pythonidae). We selected this genus for two reasons. First, the Burmese python *Python molurus* has been the focus of a collection of studies on physiological responses to feeding and fasting (Secor and Diamond, 1995; Stark and Beese, 2001; Overgaard et al., 1999; Lignot et al., 2005). With feeding, *P. molurus* experiences dramatic increases in metabolic rate, cardiac output, gastric acid production, intestinal nutrient transport and hypertrophy of the small intestine (Secor and Diamond, 1995; Secor and Diamond, 1997; Secor et al., 2000; Secor, 2003; Lignot et al., 2005). Upon the completion of digestion, these postprandial responses are reversed; metabolism is depressed, gastric acid production ceases, intestinal nutrient transport is downregulated, and the intestine atrophies. Second, of the nine genera within Pythonidae, *Python* is considered the most derived and morphologically diverse genus (Kluge, 1993). The genus *Python* is composed of ten species, four of which inhabit sub-Saharan Africa, whereas the other six inhabit southeastern Asia and Indonesia (Broadley, 1984; Kluge, 1993; Keogh et al., 2001). Three *Python* species (*P. molurus*, *P. reticulatus* and *P. sebae*) are among the largest snakes in the world (>7 m in length and 100 kg in mass), whereas *P. regius* only reaches 2 m in length and 3 kg in mass (Obst et al., 1984; Murphy and Henderson, 1997). Variation in *Python* body shape ranges from long and slender (*P. reticulatus*) to short and heavy-bodied (*P. brongersmai*) (Shine et al., 1998; Shine et al., 1999). Although it is generally assumed from anecdotal observations that all members of *Python* employ the sit-and-wait tactic of foraging and thus feed relatively infrequently, studies on gut contents suggest a more frequent feeding habit for several species (Pope, 1961; Murphy and Henderson, 1997; Shine et al., 1999).

We designed this study to determine whether differences in *Python* geographic range, body shape and potential feeding habits impact the magnitude of postprandial metabolic responses and intestinal regulation. We selected for study five species of *Python* that vary in geographic range and body shape; *P. brongersmai*, *P. molurus*, *P. regius*, *P. reticulatus* and *P. sebae*. Our objectives were to quantify for each species and compare interspecifically: (1) the profile of postprandial

metabolic response; (2) the energy expended on meal digestion and assimilation; (3) the magnitude by which intestinal function (hydrolase activity and nutrient uptake) is elevated with feeding; (4) the postprandial change in intestinal morphology and the mass of organs; and (5) the postprandial increase in intestinal performance quantified as intestinal capacity for nutrient uptake and hydrolase activity. For these five species of *Python*, we shall demonstrate both species-specific differences in postprandial responses and a general wide regulation of intestinal performance.

Materials and methods

Animals and their maintenance

The five species of this study span the geographic range and morphological diversity of the genus *Python* (Fig. 1). *Python brongersmai* Stull 1938, the blood python, inhabits eastern Sumatra and neighboring portions of Malaysia (Keogh et al., 2001). They are an extremely heavy-bodied snake [body mass to total length ratio of 8.97 ± 0.23 (mean \pm 1 s.e.m.); Fig. 1] with a body mass reaching 22 kg and a body length up to 2.5 m (Shine et al., 1999; Keogh et al., 2001). *Python molurus* L. is a large snake, up to 8 m in length and 100 kg in mass that ranges from India east into Thailand (Murphy and Henderson, 1997). *Python regius* Shaw 1802, the ball python, inhabits west-central Africa and is the smallest of the *Python* species (2 m) and is stout in body shape (Obst et al., 1984). *Python reticulatus* Schneider 1801, the reticulated python, ranges throughout southeastern Asia and Indonesia (Pope, 1961). Considered the longest snake in the world (reported lengths of 10 m), *P. reticulatus* has the most slender body shape (body mass to total length ratio of 4.53 ± 0.18 ; Fig. 1) of the *Python* species used in this study. *Python sebae* Gmelin 1789, the northern African python, occurs throughout much of the northern portion of sub-Saharan Africa and is also a large python (8 m in length and 100 kg in mass) with a body shape similar to that of *P. molurus* (Broadley, 1984). In general, *Python* species are considered to be sit-and-wait foragers that feed relatively infrequently in the wild (Pope, 1961; Murphy and Henderson, 1997). Sit-and-wait foraging snakes lie in wait in a camouflaged location from which they can ambush passing prey (Pope, 1961; Slip and Shine, 1988; Greene, 1997).

The pythons used in this study were born in captivity and purchased commercially. We housed snakes individually in 20 l plastic boxes and maintained them at 28–32°C under a photoperiod of 14 h:10 h L:D. Snakes were fed laboratory rats once every 2 weeks and had continuous access to water. To reduce potential body-size effects, we used snakes of similar mass resulting in no significant difference among the five *Python* species in body mass for either the metabolic or intestinal experiments. Prior to the start of experimentation, we withheld food from snakes for a minimum of 30 days to ensure that they were postabsorptive. *Python molurus* has been found to complete digestion within 10–14 days after feeding (Secor and Diamond, 1995). All individual snakes used in this study were between 18 and 24 months old, with body masses of

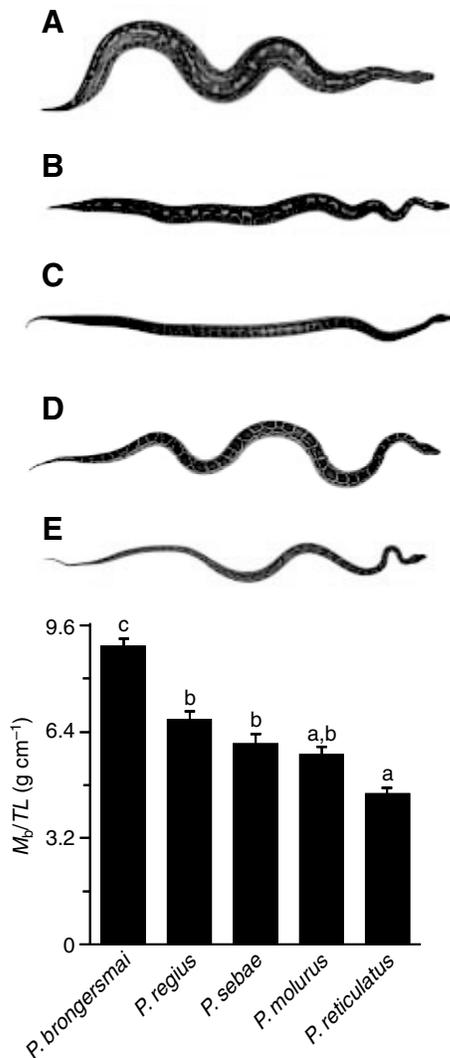


Fig. 1. Photographs and relative body shape (body mass, M_b /total length, TL) of the five *Python* species used in this study. (A) *P. brongersmai*, (B) *P. regius*, (C) *P. sebae*, (D) *P. molurus*, (E) *P. reticulatus*. Note the significant variation in body shape from the short and stout *P. brongersmai* to the long and slender *P. reticulatus*. In the histogram, letters above bars that are different denote significant ($P < 0.05$) differences between means, as determined from *post hoc* pairwise comparisons.

studied *P. brongersmai*, *P. molurus*, *P. regius*, *P. reticulatus* and *P. sebae* averaging 806 ± 51 ($N=9$), 760 ± 47 ($N=7$), 707 ± 71 ($N=10$), 757 ± 49 ($N=10$) and 759 ± 47 ($N=10$) g, respectively. Animal care and experimentation were conducted under protocols approved by the University of Alabama Institutional Animal Care and Use Committee.

Measurements of postprandial metabolic response

We quantified the postprandial metabolic response of each species by measuring rates of oxygen consumption (\dot{V}_{O_2}) from snakes fasted for 30 days and following feeding. Measurements were made using closed-system respirometry as described

(Secor and Diamond, 1997; Secor, 2003). Each metabolic trial began by measuring \dot{V}_{O_2} of fasted snakes twice a day (morning and evening) for up to 6 days and assigning the lowest measured \dot{V}_{O_2} of each snake over that time period as its standard metabolic rate (SMR). Snakes were then fed a meal consisting of one to three rats equaling $25.0 \pm 0.0\%$ of their body mass and metabolic measurements were resumed at 12-h intervals for 3 days and at 24-h intervals thereafter for 11 more days. At 5-day intervals during metabolic measurements, snakes were removed from their chambers, weighed, provided with water, and then returned back to their chambers.

We characterized the postprandial metabolic response of meal break down, absorption and assimilation of each snake by quantifying the following six variables as described by Secor and Faulkner (Secor and Faulkner, 2002): (1) SMR, the lowest measured \dot{V}_{O_2} prior to feeding; (2) peak \dot{V}_{O_2} , the highest recorded \dot{V}_{O_2} following feeding; (3) factorial scope of peak \dot{V}_{O_2} , calculated as peak \dot{V}_{O_2} divided by SMR; (4) duration, the time after feeding that \dot{V}_{O_2} was significantly elevated above SMR; (5) SDA, specific dynamic action: the total energy expenditure above SMR over the duration of significantly elevated \dot{V}_{O_2} ; and (6) SDA coefficient, SDA quantified as a percentage of meal energy. We quantified SDA (kJ) by summing the extra O_2 consumed above SMR during the period of significantly elevated \dot{V}_{O_2} and multiplying that value by $19.8\ J\ ml^{-1}\ O_2$ consumed assuming that the dry matter of the catabolized rodent meal is 70% protein, 25% fat and 5% carbohydrates, and generates a respiratory quotient (RQ) of 0.73 (Gessaman and Nagy, 1988). The energy content of rodent meals was calculated by multiplying the rodent wet mass by its energy equivalent ($kJ\ g^{-1}$ wet mass) determined by bomb calorimetry. Five individual rats, each of three different size classes, were weighed (wet mass), dried, reweighed (dry mass), ground to a fine powder, and pressed into pellets. Three pellets from each individual rat were ignited in a bomb calorimeter (1266, Parr Instruments Co., Moline, IL, USA) to determine energy content ($kJ\ g^{-1}$). For each rat, we determined wet-mass energy equivalent as the product of dry mass energy content and rodent's dry mass percentage. The three rodent size classes we used weighed on average 45 ± 0.2 , 65 ± 5.0 and 150 ± 5.0 g and had an energy equivalent of 6.5 ± 0.3 , 7.0 ± 0.4 and $7.6 \pm 0.3\ kJ\ g^{-1}$ wet mass, respectively.

Tissue collection

For each species, we killed (by severing the spinal cord immediately posterior to the head) three individuals that had been fasted for 30 days and three individuals 2 days following the consumption of rodent meals equaling 25% of the snake's body mass. Following death, a mid-ventral incision was made to expose the GI tract and other internal organs, which were each removed and weighed. We emptied the contents of the stomach, small intestine and large intestine of fed snakes and reweighed each organ. The difference between full and empty weight of each organ was noted as the mass of the organ's content. Organ content mass was divided by meal mass to illustrate for each species the relative extent of digestion at 2 days postfeeding.

Intestinal nutrient uptake

In fasted and digesting snakes we measured nutrient transport rates across the intestinal brush border membrane using the everted sleeve technique as developed by Karasov and Diamond (Karasov and Diamond, 1983) and modified for snakes by Secor et al. (Secor et al., 1994) and Secor and Diamond (Secor and Diamond, 2000). The empty small intestine was everted (turned inside out), divided into equal-length thirds; each third was weighed and sectioned into 1-cm segments. Segments were mounted on metal rods, preincubated in reptile Ringer's solution at 30°C for 5 min, and then incubated for 2 min at 30°C in reptile Ringer's solution containing an unlabeled and radiolabeled nutrient and a radiolabeled adherent fluid marker (L-glucose or polyethylene glycol). We measured, from individual intestinal segments, total uptake (passive and carrier-mediated) of the amino acids L-leucine and L-proline and active carrier-mediated uptake of D-glucose. Because of the similarities between uptake rates of the proximal and middle intestinal regions, we report the average uptake rates of those two segments (noted hereafter as the anterior intestine) and those of the distal segment.

A pair of studies has shown the everted sleeve technique to severely damage the intestinal mucosa of birds, and thus question the method's ability to accurately quantify intestinal performance for those species (Starck et al., 2000; Stein and Williams, 2003). To determine whether the method has any damaging effects on python intestine, we compared sets of intestinal segments removed from the proximal region of the small intestine of fed *P. molurus*, *P. reticulatus* and *P. sebae* at two stages of the everted sleeve protocol; prior to eversion and after everted tissues were incubated at 30°C in unstirred reptile Ringers for 5 min and in stirred reptile Ringers for 2 min. We prepared each intestinal segment for light microscopy (described below) and examined cross sections of the intestine for damage to the mucosal layer.

For each of these three pythons, everting, mounting and incubating intestinal segments did not damage the mucosal layer. Between the two stages of the procedure, we observed no significant difference (all $P > 0.47$) in villus length ($N=20$ per stage of procedure) for these three species. In contrast to some birds, the everted sleeve can be performed without damaging the intestinal mucosa of pythons, as well as the mucosa of lizards and anurans (Secor, 2005b; Tracy and Diamond, 2005).

Brush border enzyme activity

From each intestinal third we measured the activity of the brush border-bound hydrolase, aminopeptidase-N (EC 3.4.11.2) following the procedure of Wojnarowska and Gray (Wojnarowska and Gray, 1975). Aminopeptidase-N cleaves NH_2 -terminal amino acid residues from luminal oligopeptides to produce dipeptides and amino acids that then can be absorbed by the small intestine (Ahnen et al., 1982). From 1-cm segments, scraped mucosa was homogenized in PBS (1:250 dilutions) on ice. Activity of aminopeptidase-N was measured using leucyl- β -naphthylamide (LNA) as the substrate and *p*-hydroxymercuribenzoic acid to inhibit nonspecific cytosol

peptidases. Absorbance of the product resulting from the hydrolysis of LNA was measured spectrophotometrically (DU 530, Beckman Coulter, Inc., Fullerton, CA, USA) at 560 nm and compared to a standard curve developed with β -naphthylamine. Enzyme activities were quantified as μmol of substrate hydrolyzed per minute per gram of protein. Protein content of the homogenate was determined using the Bio-Rad Protein Assay kit based on the method of Bradford (Bradford, 1976).

Intestinal morphology and organ masses

We quantified the effects of feeding on small intestinal morphology by measuring intestinal mass, intestinal length, mucosa and muscularis/serosa thickness and enterocyte dimensions from fasted and fed snakes. Immediately following the removal and flushing of the small intestine, we measured its wet mass and length. From the middle region of the small intestine, a 1-cm segment was fixed in 10% neutral-buffered formalin solution, embedded in paraffin and cross sectioned (6 μm). Several cross sections were placed on a glass slide and stained with Hematoxylin and Eosin. We measured mucosa and muscularis/serosa thickness and enterocyte dimensions from individual cross sections using a light microscope and video camera linked to a computer and image-analysis software (Motic Image Plus, Richmond, British Columbia, Canada). We calculated the average thickness of the mucosa and muscularis/serosa from ten measurements taken at different positions of the cross section. Likewise, we averaged the height and width of ten enterocytes measured at different positions of the cross section and calculated their volume based on the formula for a cube (enterocyte width² \times height). To assess postprandial effects on the mass of other organs, we weighed the wet mass of the heart, lungs, liver, empty stomach, pancreas, empty large intestine and kidneys immediately upon their removal from snakes. Each organ was dried at 60°C for 2 weeks and then reweighed for dry mass.

Small intestinal capacity

For each nutrient we quantified the intestine's total uptake capacity (reported as $\mu\text{mole min}^{-1}$) by summing together the product of segment mass (mg) and mass-specific rates of nutrient uptake ($\text{nmole min}^{-1} \text{mg}^{-1}$) for the proximal, middle and distal segments. Likewise, we quantified total small intestinal capacity for aminopeptidase-N activity by summing the products of mucosa segment mass (mg) times segment aminopeptidase-N activity, calculated as μmol of substrate hydrolyzed per minute per mg of mucosa. Mucosa mass was calculated from the mass of scraped mucosa from a 1-cm segment of intestine and multiplying that mass by segment length.

Statistical analyses

For each metabolic trial we used repeated-measures design analysis of variance (ANOVA) to test for significant effects of time (before and after feeding) on \dot{V}_{O_2} . Additionally, we used *post hoc* pairwise mean comparisons (Tukey-Kramer

Table 1. *Metabolic parameters measured in five Python species*

Variable	<i>P. brongersmai</i>	<i>P. molurus</i>	<i>P. regius</i>	<i>P. reticulatus</i>	<i>P. sebae</i>	<i>F</i>	<i>P</i>
<i>N</i>	6	7	6	8	8	–	–
Body mass (g)	763±72	719±38	715±107	730±66	706±37	0.110	0.978
Meal mass (g)	190±20	180±9	178±26	183±17	176±10	0.430	0.758
Meal size (% of body mass)	25.0±0.0	25.1±0.1	25.0±0.2	25.0±0.0	24.9±0.2	0.300	0.902
SMR (ml h ⁻¹)	16.5±1.2 ^a	18.4±1.3 ^{a,b}	16.4±3.5 ^a	24.1±1.7 ^c	22.0±1.4 ^{b,c}	12.7	< 0.0001
SMR (ml g ⁻¹ h ⁻¹)	0.021±0.001 ^a	0.026±0.001 ^{a,b}	0.022±0.002 ^a	0.034±0.001 ^c	0.030±0.001 ^{b,c}	14.0	<0.0001
Peak \dot{V}_{O_2} (ml h ⁻¹)	184±18 ^a	265±18 ^b	156±28 ^a	253±20 ^b	253±10 ^b	21.7	<0.0001
Peak \dot{V}_{O_2} (ml g ⁻¹ h ⁻¹)	0.241±0.011 ^a	0.374±0.034 ^b	0.216±0.012 ^a	0.347±0.009 ^b	0.349±0.009 ^b	15.8	<0.0001
Scope of peak \dot{V}_{O_2} (peak \dot{V}_{O_2} /SMR)	11.3±0.6 ^a	14.5±1.0 ^b	9.9±0.7 ^a	10.4±0.4 ^a	11.7±0.5 ^a	7.54	<0.0003
Duration (days)	8	6	8	7	6	–	–
SDA (kJ)	322±28	317±20	326±63	340±24	347±13	2.56	0.059
SDA (kJ kg ⁻¹)	422±18	447±37	447±22	474±20	496±16	1.43	0.248
SDA (% of ingested; kJ)	23.1±1.0	24.5±2.0	25.1±1.2	25.6±1.1	27.3±0.9	1.25	0.312

SMR, standard metabolic rate; \dot{V}_{O_2} , rate of oxygen consumption; SDA, specific dynamic action.

Values are presented as means ± 1 s.e.m.

For variables with significant *P* values, different superscript letters denote significant (*P*<0.05) differences between means of the five species as determined from *post hoc* pairwise comparisons.

procedure) to determine when post feeding \dot{V}_{O_2} was no longer significantly different from SMR, and to identify significant differences in \dot{V}_{O_2} between sampling times. To test for species effects on metabolic variables, we used ANOVA for mass-specific rates and analysis of covariance (ANCOVA), with body mass as the covariate, for whole-animal measurements. Significant ANOVA and ANCOVA results were followed by *post hoc* comparisons to identify significant differences between species.

A repeated-measures design ANOVA and *post hoc* comparisons were employed to test for positional effects (proximal, middle and distal regions of the small intestine) on nutrient uptake rates and aminopeptidase-N activities. We used ANOVA to determine the postfeeding effects on nutrient uptake rates and aminopeptidase-N activity, and ANCOVA (body mass as the covariate) to test for postfeeding changes in total small intestinal capacity for nutrient uptake and aminopeptidase-N activity. Likewise, we used ANCOVA (body mass as the covariate) to test for postfeeding effects on intestinal mass, length and morphology, and the wet and dry masses of other organs. Species differences in intestinal morphology were also explored by ANCOVA and *post hoc* comparisons. We designate the level of significance as *P*<0.05 and report mean values as means ± 1 s.e.m.

Results

Metabolic response to feeding

Body mass, meal mass, and relative meal size (% of body mass) did not differ significantly in the five species (Table 1). By contrast, SMR (as ml O₂ h⁻¹ or ml O₂ g⁻¹ h⁻¹) varied significantly (all *P*<0.0001) among species as *P. reticulatus* and *P. sebae* had a significantly (both *P*<0.0013) higher SMR than *P. brongersmai* and *P. regius* (Table 1). All species exhibited significant (all *P*<0.0001) variation in \dot{V}_{O_2} , both pre

and postfeeding, with \dot{V}_{O_2} increasing significantly (all *P*<0.0002) for each species within 12 h after feeding (Fig. 2). Oxygen consumption continued to increase before peaking at 1.5 days postfeeding, at rates that ranged between 9.9- and 14.5-fold higher than SMR (Table 1). We found peak \dot{V}_{O_2} , as well as the scope of peak \dot{V}_{O_2} , to vary significantly (all *P*<0.0003) among the five pythons (Table 1). The three larger species (*P. molurus*, *P. reticulatus* and *P. sebae*) showed significantly (all *P*<0.0018) higher peak rates than the two smaller species (*P. brongersmai* and *P. regius*). *Python molurus* had the largest scope of peak \dot{V}_{O_2} (14.5±1.0), which was significantly (all *P*<0.032) greater than the scopes exhibited by the other four species (Table 1). For these five pythons, the duration of significantly elevated metabolic rates lasted from 6 to 8 days (Table 1).

The summed energy expended on digestion, absorption and assimilation (SDA) did not differ among the five species when calculated either as kJ or kJ g⁻¹ (Table 1). Given the lack of variation in SDA and meal size (and thus energy), the SDA coefficient (SDA as a percentage of meal energy) likewise did not differ significantly among the five species, averaging 25.3±0.6% (Table 1).

Digestion rates

By 2 days postfeeding, 59%, 48%, 56%, 34% and 42% of the original rodent meals remained in the stomachs of *P. brongersmai*, *P. molurus*, *P. regius*, *P. reticulatus* and *P. sebae*, respectively (Fig. 3). The relative amount of the meal found in the stomach differed significantly (*P*=0.002) among the five *Python* species. *Python brongersmai* had a larger percentage of its meal still within its stomach compared to *P. reticulatus* and *P. sebae*, and *P. regius* retained more of its meal than *P. reticulatus*. Mass of small intestinal content did not significantly vary among species, averaging 9.8±1.0% of original meal mass (Fig. 3).

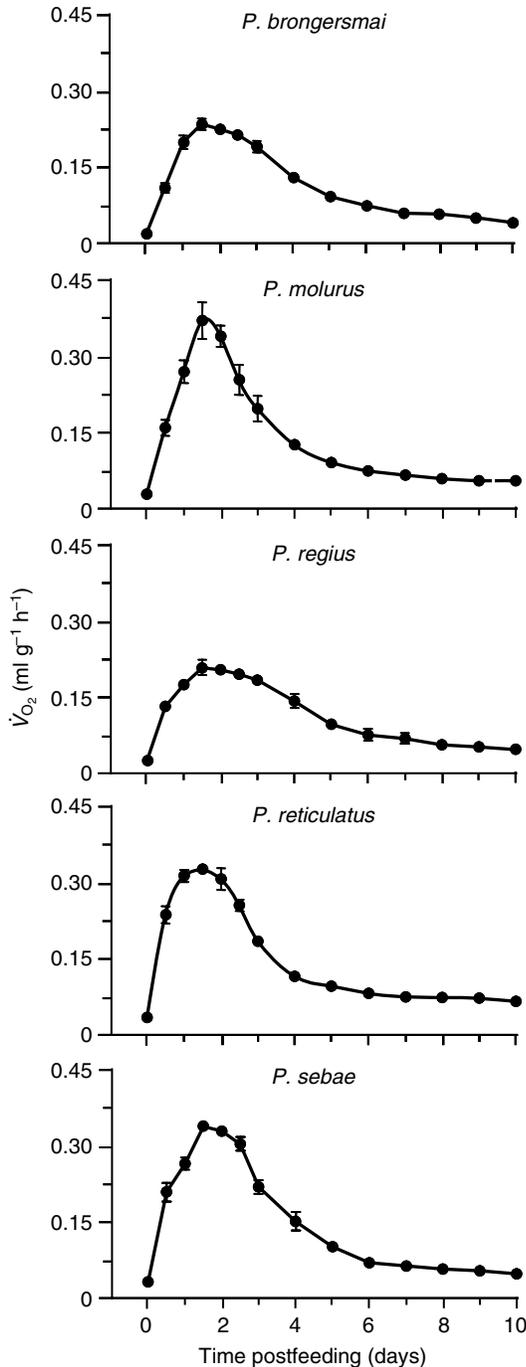


Fig. 2. Mean rates of oxygen consumption (\dot{V}_{O_2}) prior to day 0 and up to 10 days following the consumption of rodent meals equaling 25% of the snake body mass for *Python brongersmai*, *P. molurus*, *P. regius*, *P. reticulatus* and *P. sebae* ($N=6-8$ for each species). In this and the following figures, error bars indicate ± 1 s.e.m. and are omitted if the s.e.m. is smaller than the width of the symbol used for the mean value. Note the rapid increase in \dot{V}_{O_2} following the consumption of a meal and a slower return to fasting rates by days 6–9.

Intestinal nutrient uptake

For each of the five *Python* species, there was no significant difference in snout–vent length, total length, or body mass

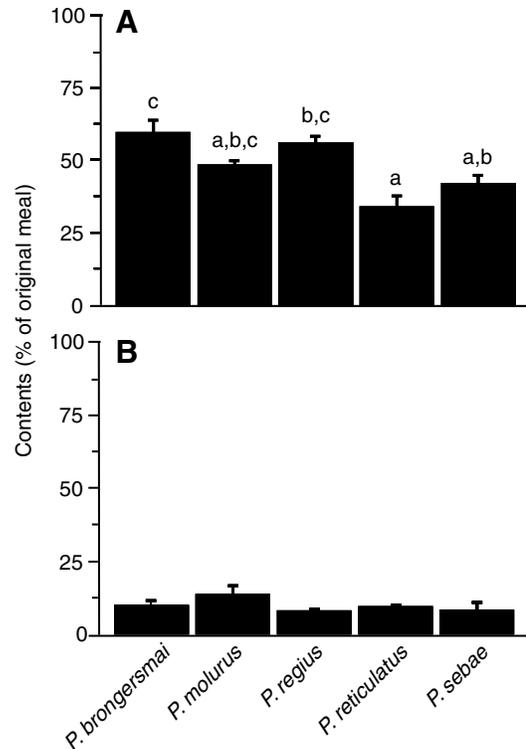


Fig. 3. Percentage of ingested meal that was recovered within the stomach (A) and small intestine (B) of *Python brongersmai*, *P. molurus*, *P. regius*, *P. reticulatus* and *P. sebae* 2 days after the consumption of rodent meals equaling 25% of their body mass ($N=3$ for each species). There was significant variation in the percentage of the ingested meal remaining within stomachs among the species, as both *P. brongersmai* and *P. regius* had more of the meal still remaining than *P. reticulatus* and *P. sebae*. By contrast, there was no variation in the percentage of the ingested meal left in the small intestine. In A, letters above bars that are different denote significant ($P<0.05$) differences between means as determined from *post hoc* pairwise comparisons.

between fasted and fed snakes. For 13 of the 30 cases (five species, two treatments, three nutrients), intestinal position had a significant (all $P<0.049$) effect on nutrient uptake rates, as uptake rates of the proximal segment were significantly greater than rates of the distal segment. Combining all fasted and fed pythons, uptake rates of L-leucine, L-proline and D-glucose declined by an average of 16%, 34% and 64%, respectively, from the proximal to distal segment.

Python molurus, *P. regius*, *P. reticulatus* and *P. sebae* each experienced significant (all $P<0.018$) postfeeding increases in L-leucine, L-proline and D-glucose uptake rates by the anterior portion of the small intestine (Fig. 4). For these four pythons, uptake rates of L-leucine increased by 6.4-, 2.9-, 5.9- and 3.4-fold, of L-proline by 4.5-, 3.5-, 5.1- and 3.1-fold, and of D-glucose by 7.7-, 27.1-, 13.6- and 16.1-fold, respectively. By contrast, *P. brongersmai* lacked any significant postfeeding increase in amino acid uptake by the anterior small intestine, though did significantly ($P<0.0014$) upregulate anterior intestinal uptake of D-glucose, by 40-fold (Fig. 4).

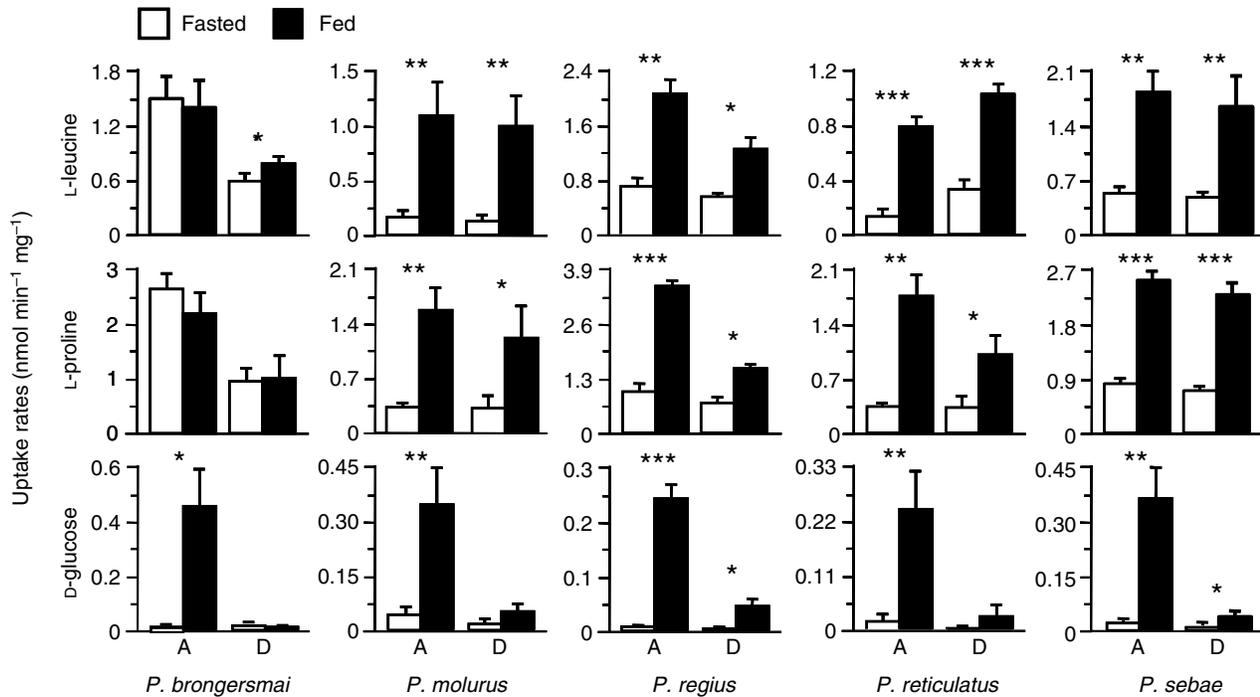


Fig. 4. Uptake rates of the amino acids L-leucine and L-proline and of the sugar D-glucose by the anterior (A) and distal (D) portions of the small intestine of fasted (following a 30-day fast, open bars) and fed (2 days postfeeding, solid bars) *Python brongersmai*, *P. molurus*, *P. regius*, *P. reticulatus* and *P. sebae*. All species (with the exception of *P. brongersmai* for L-leucine and L-proline) showed significant postprandial increases in nutrient uptake by the anterior small intestine and in many cases by the distal intestine. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Significant postprandial upregulation of nutrient transport occurred in the distal small intestine of all five species (Fig. 4). Significant postprandial uptake of L-leucine occurred in *P. brongersmai*, *P. molurus*, *P. regius*, *P. reticulatus* and *P. sebae* by factors of 1.3-, 7.6-, 2.2-, 3.1- and 3.4-fold, respectively; of L-proline in *P. molurus*, *P. regius*, *P. reticulatus* and *P. sebae* by 3.7-, 2.1-, 3.0- and 3.2-fold, respectively; and of D-glucose in *P. regius* by 21.5-fold.

Intestinal aminopeptidase-N activity

Aminopeptidase-N activity varied significantly (all $P < 0.027$) depending on intestinal positions in fed *P. brongersmai* and *P. sebae*, as activity was significantly greater in the proximal compared to the distal region. For each species studied, aminopeptidase-N activity of the anterior intestine was significantly (all $P < 0.033$) greater in fed snakes than in fasted snakes (Fig. 5). On average, among the five species, aminopeptidase-N activity of the anterior small intestine increased by 4.4-fold with feeding. Three species, *P. brongersmai*, *P. molurus* and *P. reticulatus*, also experienced significant (all $P < 0.0095$) upregulation of aminopeptidase-N activity in the distal intestine (Fig. 5).

Postprandial changes in intestinal morphology and organ mass

There was a significant (all $P < 0.044$) postprandial increase in small intestinal mass in all of the five python species

(Table 2). On average, the small intestine of these pythons doubled in mass within 2 days after feeding (Fig. 6). For *P. reticulatus* only, the postprandial increase in small intestinal mass was also accompanied by a significant ($P = 0.038$) increase (17%) in small intestinal length (Fig. 6). For fasted *P. molurus*, *P. regius* and *P. sebae* and for all five species postfeeding, there was significant (all $P < 0.046$) variation in the wet mass of intestinal segments. In each case, the proximal segment was significantly (all $P < 0.045$) heavier (by $100 \pm 13\%$) than the distal segment.

For each *Python* species, the thickness of the combined muscularis and serosa layers did not differ significantly between fasted and fed snakes (Fig. 7). By contrast, the mucosal layer increased significantly (all $P < 0.017$) in thickness postfeeding in all five species, increasing on average by $85 \pm 10\%$ (Fig. 7). The thickening of the mucosa reflects the postprandial lengthening of the villi, which was largely due to the hypertrophy of the epithelial cells, the enterocytes. For all species, enterocyte height did not change with feeding, whereas enterocyte width did increase significantly (all $P < 0.036$) by 27%, to 59% (Fig. 7). Applying the equation for a cube, we calculated enterocyte volume for fasted and fed snakes, and observed a 37%, 27%, 43%, 42% and 59% postprandial increase in enterocyte volume for *P. brongersmai*, *P. molurus*, *P. regius*, *P. reticulatus* and *P. sebae*, respectively (Fig. 7).

For all five species, feeding generated a significant (all $P < 0.041$) increase in the wet mass (and in most cases the dry

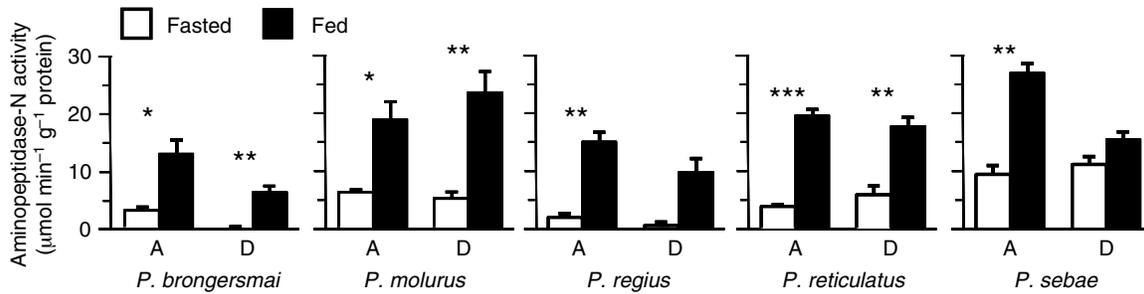


Fig. 5. Aminopeptidase-N activity of the anterior (A) and distal (D) portions of the small intestine of fasted (following a 30-day fast, open bars) and fed (2 days postfeeding, solid bars) *Python brongersmai*, *P. molurus*, *P. regius*, *P. reticulatus* and *P. sebae*. There were significant postprandial increases in aminopeptidase-N activity of the anterior small intestine in all species, and the distal small intestine in three species; * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

mass) of the liver and kidneys (Table 2). At 2 days postfeeding, liver and kidney wet masses had increased by $68.3\pm 8.7\%$ and $62.8\pm 10.9\%$, respectively, in the five species. Additionally, postprandial changes in organ mass included a significant decrease ($P=0.005$) in wet mass of the gall bladder in *P. reticulatus* and increase ($P=0.022$) in pancreatic wet mass in *P. brongersmai* (Table 2).

Intestinal digestive capacity

The combined postprandial increase in small intestinal mass and mass-specific rates of brushborder function underlie the dramatic upregulation of intestinal performance that each of these pythons experience with feeding. When summed for the full length of the small intestine, each species' capacity to transport nutrients increased significantly (all $P<0.036$) with feeding (Fig. 8). When averaged across the three measured nutrients, total intestinal uptake capacity increased with feeding by factors of 13-, 15-, 20-, 12- and 15-fold for *P. brongersmai*, *P. molurus*, *P. regius*, *P. reticulatus* and *P. sebae*, respectively. When averaged across the five species, we found L-leucine and L-proline uptake capacities to increase by similar magnitudes, 7.6-fold and 6.5-fold, respectively, with feeding. More dramatic is the concurrent upregulation of D-glucose uptake capacity, averaging 31.2-fold among the five species.

In similar fashion, as a result of combined intestinal hypertrophy and postfeeding increases in aminopeptidase-N activity, all five *Python* species experienced significant (all $P<0.006$) postfeeding increases in total intestinal aminopeptidase-N capacity (Fig. 8). By 2 days postfeeding, *P. brongersmai*, *P. molurus*, *P. regius*, *P. reticulatus* and *P. sebae* had increased their intestinal aminopeptidase-N capacity by 10.5-, 7.3-, 8.5-, 8.2- and 5.6-fold, respectively.

Discussion

Although differing in body shape, adult size and geographic distribution, members of the genus *Python* experience significant, and in many cases dramatic, postprandial responses in metabolism, organ mass and intestinal performance. As an apparent adaptive feature of their infrequent feeding habits

Python brongersmai, *P. molurus*, *P. regius*, *P. reticulatus* and *P. sebae* downregulate intestinal performance with fasting and consequently rapidly upregulate gut performance with feeding. In the ensuing discussion, we shall address in turn the postprandial metabolic, functional and trophic responses of *Python*, the proximate mechanisms underlying the regulation of their intestinal performance, the adaptive significance of their digestive physiology, and several questions that remain to be addressed.

Metabolic responses to feeding

All five pythons of this study exhibited the characteristic postprandial profile of metabolism, observed as a rapid postfeeding increase in \dot{V}_{O_2} that, upon peaking, declined more gradually to prefeeding rates (Fig. 2). Similar profiles of postprandial metabolism have been observed for invertebrates, fishes, amphibians, other reptiles, birds and mammals (Jobling, 1981; LeBlanc and Diamond, 1986; Carefoot, 1990; Janes and Chappell, 1995; Secor and Phillips, 1997; Hailey, 1998; Secor, 2005a). For pythons we can imagine that the large postprandial increases in their metabolic rates stem from the elevated activity of gastrointestinal and associated organs (heart, lung, kidneys, etc), and the transport and assimilation of the absorbed nutrients from their large meals. Generating the SDA response is the gastric breakdown of the intact rodent meal, the intestinal absorption of approximately 91% of ingested nutrients, and the synthesis of new body tissues equivalent to approximately 40% of ingested meal energy (Secor, 2003; Cox and Secor, 2005). For *P. molurus*, it has been estimated that gastric performance and postabsorptive protein synthesis accounts for 55% and 26.3% of SDA, respectively (Secor, 2003).

For pythons, as well as for other reptiles and amphibians, the magnitude of peak \dot{V}_{O_2} , the duration of the metabolic response, and overall SDA are affected by meal type, meal size, body temperature and body size (Secor and Diamond, 1997; Hailey, 1998; Toledo et al., 2003; Wang et al., 2003; McCue et al., 2005; Pan et al., 2005; Secor and Boehm, 2006). Therefore, interspecific comparisons of the SDA response are best made when meal type, relative meal size, body temperature and body size are standardized. To a common meal type (rats), meal size

Table 2. Body mass, snout–vent and total length, and wet and dry mass for organs removed from fasted and fed *Python* species

Variable	<i>P. brongersmai</i>		<i>P. molurus</i>		<i>P. regius</i>		<i>P. reticulatus</i>		<i>P. sebae</i>	
	Fasted	2 d.p.f.	Fasted	2 d.p.f.	Fasted	2 d.p.f.	Fasted	2 d.p.f.	Fasted	2 d.p.f.
<i>N</i>	3	3	3	3	3	3	3	3	3	3
Body mass (g)	818±136	861±42	790±109	769±29	741±110	710±85	815±99	753±48	780±112	852±25
Snout–vent length (cm)	89.3±4.5	89.5±1.3	120.7±8.8	122.3±1.9	98.1±9.2	97.8±6.2	154.3±7.1	145.5±4.8	119.3±5.8	117.0±1.0
Total length (cm)	96.2±4.8	96.5±4.8	135.0±9.5	137.0±3.1	106.7±9.6	105.7±6.9	178.2±7.8	166.7±5.2	134.7±6.6	132.7±1.3
Wet mass (g)										
Heart	2.00±0.44	2.57±0.62	2.05±0.25	2.06±0.15	1.78±0.11	1.61±0.05	1.75±0.17	1.65±0.15	1.99±0.28	2.37±0.45
Lung	5.22±1.09	5.03±0.66	6.42±0.66	6.96±0.17	5.24±0.79	4.02±0.32	5.84±0.79	6.76±1.46	5.69±0.52	6.20±0.26
Liver	11.1±2.6	20.5±1.6*	8.27±0.32	14.3±0.9*	9.17±0.76	12.8±0.4*	8.50±2.16	15.8±0.8*	11.8±1.4	18.7±1.2*
Stomach	10.1±2.1	14.6±2.3	10.1±2.1	14.3±1.2	7.09±0.80	11.9±3.0	10.5±0.8	12.1±1.1	11.9±1.6	14.7±0.5
Gall bladder	1.75±0.61	1.10±0.55	2.92±0.33	2.01±0.19	1.70±0.02	1.59±0.22	3.93±0.31	2.09±0.14*	2.62±0.29	2.50±0.35
Pancreas	0.89±0.16	1.42±0.10*	0.71±0.08	0.94±0.07	0.61±0.10	0.83±0.10	0.93±0.19	0.99±0.03	0.86±0.11	1.14±0.10
Spleen	0.06±0.01	0.11±0.04	0.10±0.01	0.14±0.02	0.06±0.00	0.06±0.00	0.09±0.00	0.05±0.03	0.17±0.04	0.18±0.05
Small intestine	10.4±2.3	23.7±1.0*	10.6±0.4	25.3±4.1*	8.34±0.77	13.7±1.9*	13.9±0.7	23.0±2.6*	10.5±1.2	24.1±0.05*
Large intestine	8.17±2.16	8.89±0.95	6.51±0.97	9.14±0.90	5.07±0.75	4.91±0.66	4.86±0.52	5.34±0.37	6.03±0.80	7.42±0.33
Kidneys	3.76±0.74	7.23±0.60*	3.49±0.45	6.14±0.09*	3.32±0.64	4.74±0.35*	3.78±0.43	5.04±0.36*	4.34±0.26	7.37±0.53*
Dry mass (g)										
Heart	0.30±0.13	0.34±0.08	0.33±0.05	0.32±0.02	0.27±0.05	0.28±0.01	0.33±0.01	0.26±0.03	0.38±0.07	0.38±0.05
Lung	0.79±0.22	0.74±0.10	1.19±0.14	1.20±0.04	0.83±0.07	0.74±0.10	1.26±0.13	1.22±0.26	1.08±0.17	1.20±0.04
Liver	5.05±1.22	6.19±1.02	3.01±0.05	3.75±0.26*	2.49±0.15	3.37±0.16*	3.10±0.35	4.27±0.18*	4.68±0.69	6.48±0.43*
Stomach	1.93±0.45	1.97±0.26	2.56±0.12	2.74±0.26	1.31±0.14	2.07±0.48	2.34±0.22	2.45±0.18	2.36±0.37	3.06±0.16
Gall bladder	0.32±0.21	0.31±0.24	0.56±0.08	0.48±0.10	0.17±0.08	0.21±0.09	0.47±0.17	0.32±0.06	0.48±0.10	0.43±0.06
Pancreas	0.11±0.04	0.25±0.01	0.14±0.01	0.12±0.00	0.13±0.02	0.15±0.02	0.22±0.05	0.21±0.01	0.19±0.03	0.23±0.02
Spleen	0.02±0.00	0.02±0.01	0.02±0.00	0.03±0.00	0.01±0.00	0.01±0.00	0.02±0.00	0.01±0.01	0.03±0.01	0.04±0.01
Large intestine	1.25±0.28	1.34±0.15	1.20±0.17	1.29±0.12	0.86±0.14	0.60±0.03	1.01±0.11	0.94±0.10	1.10±0.16	1.53±0.15
Kidneys	0.76±0.16	1.14±0.06	0.63±0.23	1.25±0.17*	0.64±0.13	0.75±0.06	1.03±0.11	1.04±0.13	0.80±0.08	1.40±0.10*

Organs were weighed immediately after removal from three fasted and three fed [2 days postfeeding (d.p.f.)] individuals of each *Python* species.

*Significant differences in organ mass between fasted and fed snakes, determined by ANCOVA ($P < 0.05$).

(25% of body mass), body temperature (30°C) and body size (mean=706–763 g), the five pythons of our study showed similar SDA responses. For each, \dot{V}_{O_2} peaked 1.5 days after feeding at 9.9- to 14.5-times SMR before declining back to prefeeding values after an additional 5–8 days (Fig. 2). Subtle interspecific differences included the lower SMR and peak \dot{V}_{O_2} of *P. brongersmai* and *P. regius*, the higher scope of peak \dot{V}_{O_2} of *P. molurus*, and the shorter duration for *P. molurus* and *P. sebae*. These differences essentially cancelled each other out in generating similar SDAs (422–496 kJ kg⁻¹) in the five species (Table 1).

In previous studies in which *P. molurus* consumed rodent meals equaling 20–25% of their body mass, snakes achieved peaks in \dot{V}_{O_2} 1–2 days postfeeding at rates between 0.25 and 0.55 ml g⁻¹ h⁻¹, a range of \dot{V}_{O_2} that encompasses our peak rates for *P. molurus*, *P. reticulatus* and *P. sebae* (Secor and Diamond, 1997; Secor et al., 2000; Overgaard et al., 2002; Wang et al., 2003). Some of the variation in reported peak \dot{V}_{O_2} can be explained by differences in relative meal size (20% versus 25% of body mass), given that postprandial peaks in \dot{V}_{O_2} increase with relative meal size (Secor and Diamond, 1997). In

a study of *P. regius*, Starck and Wimmer (Starck and Wimmer, 2005) recorded SMR and peak \dot{V}_{O_2} of 0.021 and 0.08 ml g⁻¹ h⁻¹, respectively, and a duration of the SDA response of approximately 10 days. The *P. regius* of our study had similar SMR (0.022 ml g⁻¹ h⁻¹) and response duration (8 days), however, our *P. regius* attained a higher peak \dot{V}_{O_2} (0.21 ml g⁻¹ h⁻¹).

For other infrequently feeding snakes, including the boa constrictor *Boa constrictor*, sidewinder *Crotalus cerastes*, timber rattlesnake *Crotalus horridus*, water python *Liasis fuscus*, rosy boa *Lichanura (=Charina) trivirgata*, and carpet python *Morelia spilota*, the consumption of rodent meals of 25% of their body mass likewise generated 6- to 18.5-fold increases in metabolic rate, which remained elevated for 6–8 days (Thompson and Withers, 1999; Secor and Diamond, 2000; Bedford and Christian, 2001; Zaidan and Beaupre, 2003). For *B. constrictor*, *C. cerastes* and *L. trivirgata*, SDA ranged between 357 and 670 kJ kg⁻¹, and together with the pythons of the present study, SDA coefficients vary between 18 and 33% (Secor and Diamond, 2000). By contrast, snake species that feed more frequently in the wild have more modest

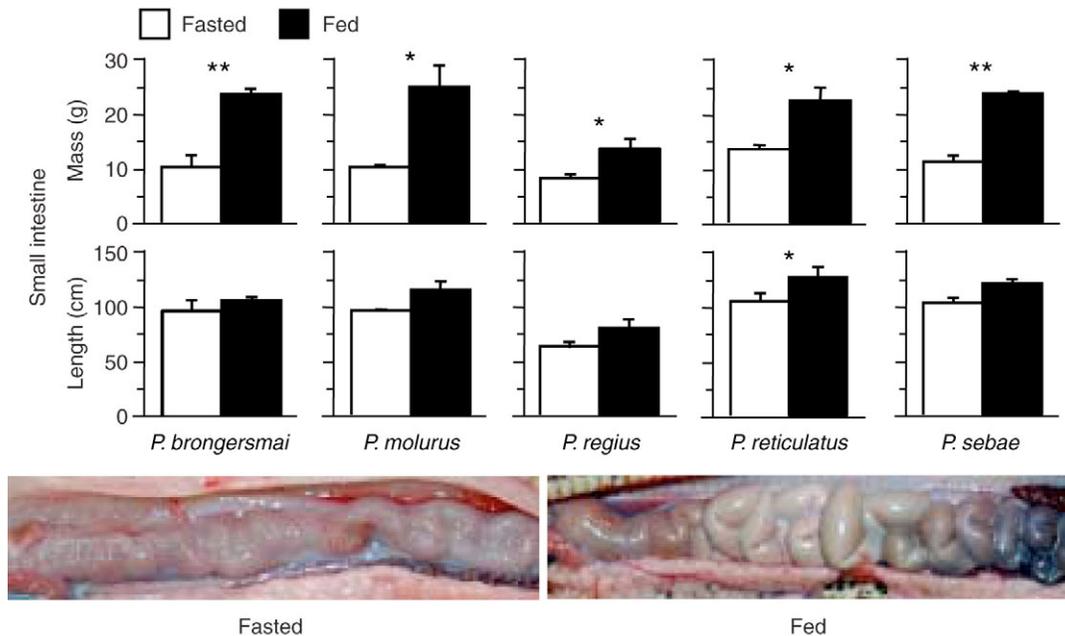


Fig. 6. Small intestinal mass and length of fasted (following a 30-day fast, open bars) and fed (2 days postfeeding, solid bars) *Python brongersmai*, *P. molurus*, *P. regius*, *P. reticulatus* and *P. sebae*. All five species had significant postfeeding increases in small intestinal mass, whereas only *P. reticulatus* showed an increase in intestinal length with feeding. The photographs of small intestines of fasted and fed *P. molurus* illustrate the postprandial trophic response. Asterisks indicate significant differences between the two states; * $P < 0.05$, ** $P < 0.01$.

SDA responses to similar size meals (20–25% of body mass), as noted by 5- to 8-fold increases in metabolism, metabolic rates that remain elevated for 3.5–5 days, SDAs of 258–309 kJ kg⁻¹, and SDA coefficients of 13–15% (Secor and Diamond, 2000; Zaidan and Beaupre, 2003; Hopkins et al., 2004; Roe et al., 2004).

Plasticity of intestinal function

There is a distinct gradient in function of the python intestine, as aminopeptidase-N activity and nutrient transport rates decline distally. A proximal to distal gradient of intestinal hydrolase activities has also been observed for amphibians, birds and mammals (McCarthy et al., 1980; Martinez del Rio, 1990; Hernandez and Martinez del Rio, 1992; Sabat et al., 2005). Similar decreases with position in mass-specific and length-specific rates of nutrient uptake have been documented for fishes, amphibians, reptiles, birds and mammals (Karasov et al., 1985; Karasov et al., 1986; Buddington and Hilton, 1987; Buddington et al., 1991; Secor and Diamond, 2000; Secor, 2005a). This phenomenon, especially evident for the active uptake of D-glucose, may best be explained by the reduction distally in functional surface area of the small intestine, a product of decreases in villus and microvillus surface area (Ferraris et al., 1989). In addition, the density of glucose transporters on the surface of the microvilli of mice (*Mus musculus*) and woodrats (*Neotoma lepida*) decreases step-wise from proximal to middle to distal regions (Ferraris et al., 1989). For pythons and other species, the positional decline in intestinal function undoubtedly

reflects a response to the distal decrease in the concentration of luminal nutrients.

For fasted and fed pythons, as for most carnivores studied, intestinal uptake rates of amino acids are significantly greater than uptake rates of D-glucose, usually by an order of magnitude (Buddington et al., 1991; Secor and Diamond, 2000; Secor, 2005a). This difference is explained by the predominance of protein within the snake's diet and the relatively small amount of dietary carbohydrates. Additionally, this difference may, in part, be due to the combined measurement of passive and active uptake of the amino acids and/or measurement of only the active transport of D-glucose. With feeding, pythons rapidly increase intestinal uptake of amino acids (with the exception of *P. brongersmai*) and D-glucose. In four of the python species, L-leucine and L-proline uptake rates increased with feeding by 2.9- to 6.4-fold, a magnitude similar to the postfeeding increases in amino acid uptake observed for *B. constrictor*, *C. cerastes* and *L. trivirgata* (Secor and Diamond, 2000). Whereas *P. brongersmai* lacked significant postfeeding increases in amino acid transport, this species, along with the other four pythons, dramatically upregulated the active transport of D-glucose by an average of 21-fold in the anterior small intestine. Likewise, significant postprandial increases in D-glucose active transport have also been documented for *B. constrictor* (5-fold), *C. cerastes* (6.8-fold) and *L. trivirgata* (4.3-fold) (Secor and Diamond, 2000).

In the anterior portion of the small intestine and in some cases in the distal portion, aminopeptidase-N activity increased significantly with feeding for each of the five pythons. This

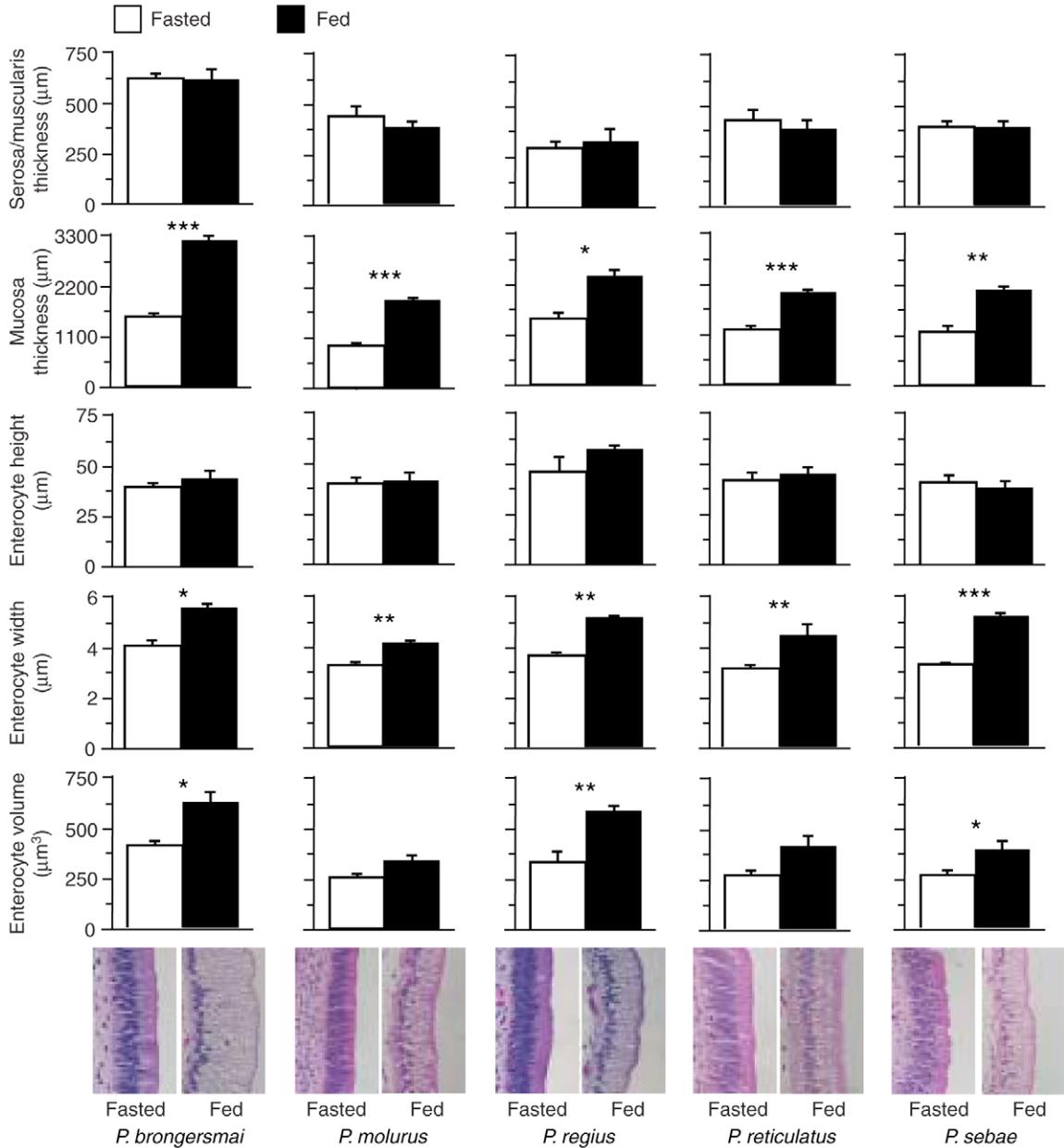


Fig. 7. Width of the intestinal muscularis/serosa and mucosa layers, and height, width and volume of intestinal enterocytes and micrographs of the intestinal epithelium of fasted (following a 30-day fast, open bars) and fed (2 days postfeeding, solid bars) *Python brongersmai*, *P. molurus*, *P. regius*, *P. reticulatus* and *P. sebae*. Note that after feeding there is a lack of change in muscularis/serosa layer thickness and enterocyte height, and the significant increase in thickness of the mucosal layer and enterocyte width and volume. Asterisks indicate significant differences between the two states; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

increase in peptidase activity is expected given both the large protein content of their meals and that the overall upregulation of intestinal function would also include increases in brush border hydrolase activity. A matched response of hydrolase activity and nutrient transport was observed in this study by the average 4.46-fold and 4.36-fold postprandial increases in anterior aminopeptidase-N activity and amino acid uptake, respectively, for four of the pythons (excluding *P. brongersmai*).

Studies on the postprandial responses of intestinal

hydrolases have generated mixed results. In rats, fasting results in an increase in intestinal peptidase activity that is reversed when the rats feed (Kim et al., 1973; Ihara et al., 2000). The Andean toad, *Bufo spinulosus*, shows no change in either intestinal aminopeptidase-N or maltase activity between fasting and feeding (Naya et al., 2005). By contrast, the pythons of this study had large postfeeding increases in the activity of intestinal aminopeptidase-N. Explanations for this continuum of regulatory responses include the increased activity of cellular peptidases in fasting rats in order to hydrolyze cellular

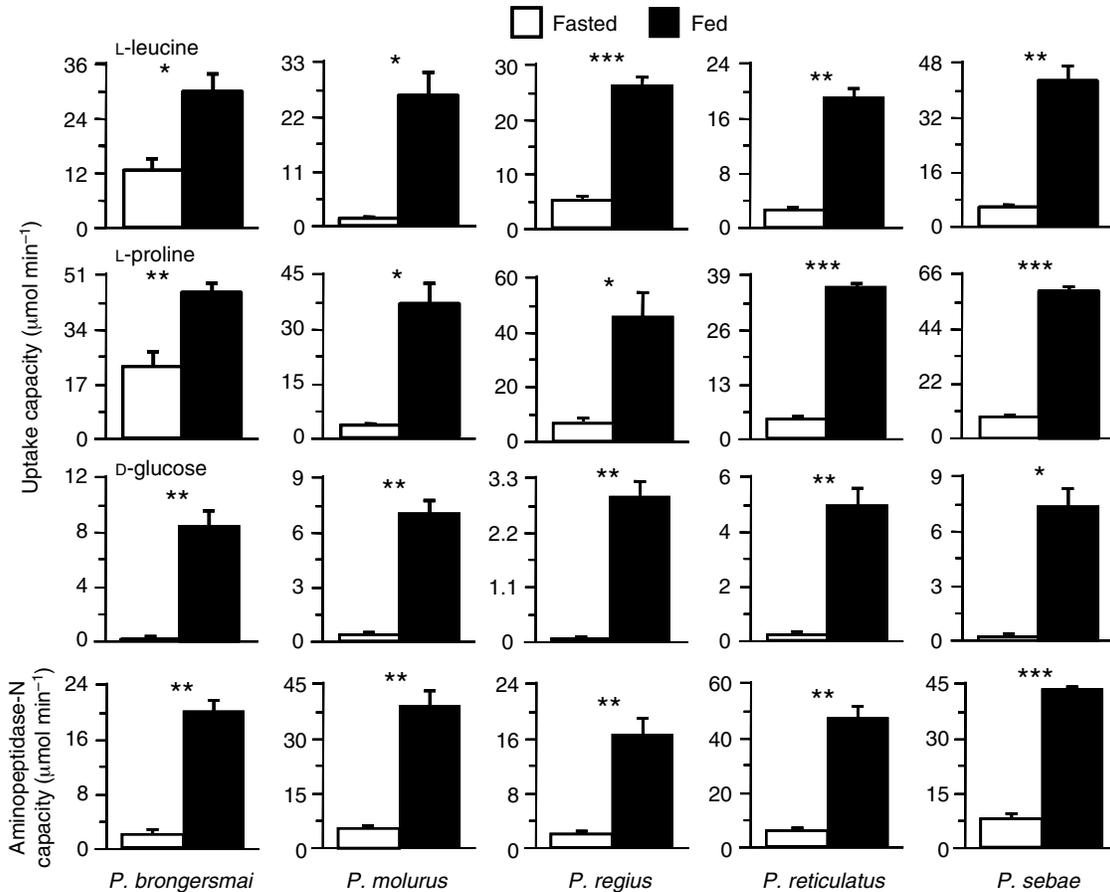


Fig. 8. Small intestine uptake capacities of L-leucine, L-proline and D-glucose, and intestinal aminopeptidase-N activity of fasted (following a 30-day fast, open bars) and fed (2 days postfeeding, solid bars) *Python brongersmai*, *P. molurus*, *P. regius*, *P. reticulatus* and *P. sebae*. After feeding, all five *Python* species significantly increased uptake of each nutrient and aminopeptidase-N activity; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

proteins as a fuel source and for gluconeogenesis, the lack of response in the Andean toad because they may feed frequently and, like other frequently feeding anurans they do not widely regulate intestinal function, and the large postfeeding increase in pythons, because as infrequent feeders they widely regulate intestinal function with each meal.

Trophic responses of the intestine and other organs

An apparent universal response to fasting is the reduction in mass (independent of changes in body mass) of the small intestine, manifested as atrophy of the intestinal epithelium (Bogé et al., 1981; Carey, 1990; Secor, 2005a). In blackcaps *Sylvia atricapilla*, small intestinal mass and villus height are reduced by 45% and 18%, respectively, after a 2-day fast, in rats by 42% and 30% after a 5-day fast, and in garter snakes *Thamnophis sirtalis* by 38% and 50% after a 4-week fast (Dunel-Erb et al., 2001; Starck and Beese, 2002; Karasov et al., 2004). Feeding rapidly reverses intestinal atrophy by triggering the hypertrophy of enterocytes, which quickly restores intestinal mass to prefeeding levels (Dunel-Erb et al., 2001; Karasov et al., 2004).

The postprandial increase in small intestinal mass observed for the five *Python* species is similar in magnitude to that

previously noted for *B. constrictor*, *C. cerastes* and *L. trivirgata*, as well as for several species of estivating anurans (Secor and Diamond, 2000; Secor, 2005b). For each of these organisms, the increase in small intestinal mass is largely attributed to the thickening of the intestinal mucosa, which results from villus lengthening, itself a product of enterocyte hypertrophy. For pythons and estivating anurans, enterocyte width and volume increase with feeding by 40–90% and 50–440%, respectively (Cramp and Franklin, 2005; Lignot et al., 2005; Secor, 2005b). In addition to enterocyte hypertrophy, cellular hyperplasia (replication) may also contribute to the postprandial increase in intestinal mass. However, for *P. molurus*, the postprandial increases in enterocyte replication are matched by a concurrent increase in apoptosis (Lignot and Secor, 2003; Lignot et al., 2005). Hence, the postprandial increase in python intestinal mass appears largely to be a product of cellular hypertrophy rather than hyperplasia.

Postprandial increases in the mass of organs, other than the small intestine, have also been observed for *B. constrictor*, *C. cerastes* and *L. trivirgata* (Secor and Diamond, 2000). For these snakes, together with pythons, feeding generates increases in liver and kidney wet masses of ~59% and ~70%, respectively. These tissues may also be experiencing cellular

hypertrophy, contributed, in part, by the accumulation of material absorbed through the gut and filtered from circulation. Other organs involved in digestion that also increase in mass with feeding, though not consistently among infrequently feeding species, include the pancreas (*P. brongersmai* and *B. constrictor*) and stomach (*C. cerastes*, *L. trivirgata* and *P. molurus*) (Secor and Diamond, 2000) (this study). We did not observe a significant postfeeding increase in heart mass in any of the pythons of this study as previously documented for *P. molurus* (Secor and Diamond, 1995; Andersen et al., 2005).

Regulatory mechanisms of intestinal performance

Each of the five *Python* species in this study exhibited the ability to widely modulate the capacity of the intestine for nutrient uptake and aminopeptidase-N activity, with feeding and fasting. For pythons, the underlying mechanisms for the regulation of intestinal performance are split between those responsible for the trophic responses and those for the functional responses of the intestinal epithelium. Within 2 days after feeding, intestinal nutrient uptake and aminopeptidase-N capacities have increased in the five pythons by 2- to 49-fold. On average, the increase in small intestinal mass and the increase in mass-specific function accounts for 21.6% and 78.4%, respectively, of the postprandial increase in intestinal capacity.

As noted earlier, the increase in small intestinal mass is due largely to hypertrophy of the epithelium enterocytes. Plausible mechanisms for enterocyte hypertrophy include the mobilization of amino acids from protein sources for enterocyte rebuilding and the absorption of luminal nutrients. Although there is no current evidence to support the former explanation, the latter explanation is well supported from observations of enterocytes of *P. molurus* filled with lipid droplets originating from the meal (Starck and Beese, 2001; Lignot et al., 2005). Our histological examinations revealed the presence of lipid droplets within enterocytes of fed snakes in all of the five python species.

There are several specific and nonspecific mechanisms by which intestinal function, independent of mass, can be regulated. First, by increasing or decreasing the specific activity of membrane transporters and enzymes. Second, by modulating the rate of synthesis and thus the density of brushborder transporters and enzymes. And third, by altering the functional surface areas of the luminal membrane without changing transporter or enzyme activity or density. The former two mechanisms have been proposed to explain shifts in nutrient transporter function with changes in diet (Buddington and Diamond, 1989; Ferraris et al., 1992). The third mechanism, involving the movement to and from the brushborder membrane of intracellular stores of membrane proteins, explains the compensatory restoration of lost function following the surgical removal of a portion of the small intestine, as the remnant intestine responds by increasing villus length (Fenyö et al., 1976; Hanson et al., 1977).

For pythons, although there is support for the second mechanism from the findings of a postprandial increase in

protein and mRNA expression of the Na⁺/glucose co-transporter (SGLT1) for *P. molurus*, we propose that it is the third mechanism that is largely responsible for the regulation of intestinal function (Secor, 2005a). Pythons, like other organisms, experience a fasting-to-feeding increase in villus length, but this would only be responsible for about an 85% increase in surface area. Unlike other organisms, pythons experience a postprandial increase in microvillus length (Secor, 2005a). All five python species in this study possessed stunted microvilli (~0.5 μm) while fasting, which increased 5-fold within 2 days after feeding (S. Secor and J-H. Lignot, unpublished data). Given that the microvilli are minute compared to the rest of the enterocyte, their increase in length contributes insignificantly to the postprandial increase in small intestinal mass. If for pythons transporter and enzymes activities and densities on the microvilli are stable from fasting to feeding, the resulting increase in microvillus surface area, resulting from the mobilization and insertion of membrane proteins from within the cell, would account for much of the upregulation of intestinal function (Secor, 2005a). This certainly would be the case for amino acid uptake and aminopeptidase-N activity, but would only account for a portion of the increase in the carrier-mediated uptake of D-glucose. We suspect that the remainder of the upregulation of D-glucose uptake is provided by the aforementioned increase in the expression and thus the density of SGLT1.

Adaptive correlates of Python digestive physiology

Our original question asked whether *Python* species possess unique differences in their digestive response that reflects species differences in biogeography, body shape and/or feeding habits, or if they exhibit, in common, the wide regulation of digestive response indicative of their infrequent feeding habits. We will first comment on species-specific differences before addressing the generality of the python digestive response. The pythons of this study are split geographically between subSaharan Africa (*P. regius* and *P. sebae*) and southeast Asia and Indonesia (*P. brongersmai*, *P. molurus* and *P. reticulatus*). A comparison of these two sets of snakes revealed no significant differences in metabolic, morphological, or functional responses to fasting or feeding with respect to geography. Interestingly, *P. molurus* from southeast Asia and *P. sebae* from Africa have numerous similarities in body morphology and in physiological responses, whereas *P. brongersmai* from Indonesia and *P. regius* from Africa likewise share similar morphologies (short-bodied) and a relatively low rate of standard metabolism.

As an index of body shape, the ratio of body mass to body length ranged from 4.53±0.18 for *P. reticulatus* to 8.45±0.56 for *P. brongersmai* (Fig. 1). Along this continuum of body shape index, we did not find any significant correlation between this ratio and metabolic responses, intestinal responses or organ masses. In looking at the two extremes of *Python* body shape, we note that the elongated *P. reticulatus* possessed the highest SMR and largest SDA, whereas the stout *P. brongersmai* exhibited the smallest upregulation of amino acid

uptake capacity and the largest increase in aminopeptidase-N capacity. It is interesting that despite having the shortest SVL, *P. brongersmai* small intestines are similar in length to those of *P. molurus*, *P. reticulatus* and *P. sebae*. Snake small intestines are arranged in a serpentine fashion and therefore are much longer than the length of body cavity that they occupy. For *P. brongersmai*, the ratio of small intestinal length to body cavity length occupied by the small intestine (13.1 ± 1.6) was significantly greater than that of the other four species (6.1 ± 0.4).

Although data on feeding habits for these five species is scant, the existing anecdotal and scientific reports suggest that *Python* species utilize an ambush foraging strategy to feed chiefly upon birds and mammals (Pope, 1961; Murphy and Henderson, 1997). In southern Sumatra, *P. reticulatus* consume mostly rats as juveniles, graduating to monkeys, wild pigs and small deer as adults (Shine et al., 1998). On oil palm plantations in northeastern Sumatra, juvenile and adult *P. brongersmai* feed almost exclusively on rats (Shine et al., 1999). In these two previous studies, it was found that 50% of collected *P. reticulatus* had the remains of a meal within their gut (stomach, small and large intestines), whereas 78% of collected *P. brongersmai* had food items in their guts. This difference in the occurrence of gut contents may suggest that *P. brongersmai* feeds more frequently than *P. reticulatus*, but the presence of food in the gut may not always be a good predictor of feeding frequency for pythons. Observed in captivity, *Python* species vary tremendously in the duration that they retain fecal matter within their large intestine, from 1–2 weeks for *P. reticulatus* to 2–4 months for *P. brongersmai* (B. Ott, personal observations). Apparently, retention of fecal matter for extended lengths of time has also been observed for other short and stout, sit-and-wait foraging snakes (Lillywhite et al., 2002). Therefore, these five species may possess similar feeding frequencies, and hence similar magnitudes of postprandial responses.

Stepping back from the interspecific variation in *Python* postprandial physiological responses, each of the five *Python* species was observed to significantly regulate intestinal performance with each meal. The downregulation of intestinal performance with fasting is proposed to be an adaptation for organisms that predictably experience long intervals between meals (Secor, 2001; Secor, 2005a). For fasting animals relying solely upon stored energy to meet metabolic demands, any trait that reduces daily energy expenditure would be favored by natural selection. Given the high maintenance cost of the gastrointestinal epithelium, due in part to its high rate of cell turnover (Johnson, 1987), its downregulation in structure and function during fasting would therefore reduce overall energy expenditure. For pythons and other snakes that naturally experience long fasts between meals, this reduction in gut maintenance is manifested in part as a lowering of their SMR. On average, SMR of the pythons of this study and other infrequently feeding snakes is 48% lower than the SMR of frequently feeding snakes that do not significantly downregulate intestinal performance with fasting (Fig. 9).

The conclusions we draw from this study are: (1) members of the genus *Python* respond to each meal with large increases in

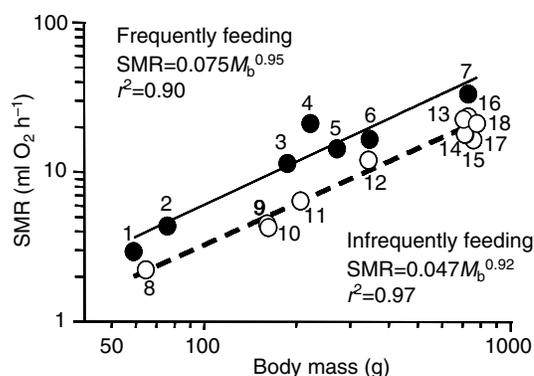


Fig. 9. Standard metabolic rate (SMR) at 30°C plotted against body mass M_b for seven species of frequently feeding snakes and 11 species of infrequently feeding snakes. Interspecific allometric equations were generated from least-squares regression analysis of data from Secor and Diamond (Secor and Diamond, 2000), this study, and our unpublished observations. Numbers signify the following species: 1, *Thamnophis marcianus*; 2, *Thamnophis sirtalis*; 3, *Lampropeltis getula*; 4, *Coluber constrictor*; 5, *Masticophis flagellum*; 6, *Nerodia rhombifer*; 7, *Pituophis melanoleucus*; 8, *Morelia spilota*; 9, *Crotalus cerastes*; 10, *Lichanura trivirgata*; 11, *Acrantophis dumerili*; 12, *Boa constrictor*; 13, *Python sebae*; 14, *P. regius*; 15, *P. molurus*; 16, *P. reticulatus*; 17, *P. brongersmai*; 18, *Eunectes murinus*. Note that across the range of body masses compared, infrequently feeding snakes have SMR that are almost 50% less than those of frequently feeding species.

metabolic rate, intestinal hypertrophy, and the elevation of intestinal function; (2) the subtle interspecific variation in physiological and morphological responses among the five species of *Python* are not associated with either geography (Africa vs Asia) or body shape (stout vs elongated body shape); and (3) as previously described for *P. molurus*, these five species share the adaptive capacity to widely regulate gastrointestinal performance with feeding and fasting. Although not studied, we predict that the other five species of *Python* (*P. anchietae*, *P. breitensteini*, *P. curtus*, *P. natalensis* and *P. timoriensis*) likewise up- and downregulate gastrointestinal performance with each meal.

Further inquiry in snake digestive physiology

Seldom is a study undertaken that does not generate new questions, alternative hypotheses, and further explorations. While examining the metabolism, morphology and postfeeding responses of these five python species, we identified two further areas warranting further investigation.

(1) *What is the significance of interspecific differences in postfeeding responses and morphology among python species?* Although we have downplayed the importance of those differences in the overall regulation of digestive performance, they are worthy of further attention. Consider *P. brongersmai*; why did this species not downregulate amino acid uptake while fasting as it did D-glucose uptake and aminopeptidase-N activity, and why does it possess such a long intestine for its body length? Additional information on the feeding habits of this species,

repeating the study, and studying the postprandial responses of sister taxa (*P. breitensteini* and *P. curtus*) may explain (or refute) this species' lack of amino acid transport regulation. The small intestine of *P. brongersmai* is similar in length to that of the three longest pythons (*P. molurus*, *P. reticulatus* and *P. sebae*), although relative to body length, it is twice as long. Is this trait unique for *P. brongersmai*, or is intestinal length conserved with respect to body mass, and it is *P. regius* that possess the uniquely short small intestine (averaging 66% the length of the small intestine of the other four species)?

(2) *Is the wide regulation of gastrointestinal performance an inherent plesiomorphic character of lineages of infrequently feeding snakes?* In the present study we were not surprised to find that *Python* species widely regulate intestinal performance, given their infrequent feeding habits and the large postprandial responses known for *P. molurus*. In the family Pythonidae there are approximately 27 species within eight genera, and in the sister family Boidae there are approximately 40 species within 11 genera. Members of these two families are generalized as sit-and-wait foragers that feed relatively infrequently (Greene, 1997), and therefore hypothetically they all possess the plesiomorphic trait of widely regulating digestive performance with each meal. Alternatively, given the much broader variation in biogeography, body shape, ecology and feeding habits among all pythons and boas (compared to just *Python*), there may be species that lack this trait, and, like frequently feeding colubrid snakes, only modestly regulate intestinal function between meals. Candidate species for the modest regulation of digestive function could include arboreal species of the genus *Morelia* (Pythonidae) and *Corallus* (Boidae) that are extremely long and slender and may feed more frequently in the wild (Henderson, 2002). Studies on these genera, and others, may reveal the independent evolution of the narrow regulation of gastrointestinal performance within lineages of snakes that largely regulate gut performance. A further expansion of this inquiry would include studies on members of adjoining basal lineages (Loxocemidae and Xenopeltidae) and those (Acrochordidae, Bolyeriidae, Tropidophiidae and Xenophidiidae) positioned phylogenetically between pythons and boas and the family Viperidae, which includes the wide regulating *Crotalus cerastes* (Secor et al., 1994; Pough et al., 2004). These studies would be the next step in elucidating the evolutionary pattern of digestive response among snakes.

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