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RESEARCH ARTICLE

Selected regulation of gastrointestinal acid–base secretion and tissue metabolism for the diamondback water snake and Burmese python

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SUMMARY

Snakes exhibit an apparent dichotomy in the regulation of gastrointestinal (GI) performance with feeding and fasting; frequently feeding species modestly regulate intestinal function whereas infrequently feeding species rapidly upregulate and downregulate intestinal function with the start and completion of each meal, respectively. The downregulatory response with fasting for infrequently feeding snakes is hypothesized to be a selective attribute that reduces energy expenditure between meals. To ascertain the links between feeding habit, whole-animal metabolism, and GI function and metabolism, we measured preprandial and postprandial metabolic rates and gastric and intestinal acid–base secretion, epithelial conductance and oxygen consumption for the frequently feeding diamondback water snake (*Nerodia rhombifer*) and the infrequently feeding Burmese python (*Python molurus*). Independent of body mass, Burmese pythons possess a significantly lower standard metabolic rate and respond to feeding with a much larger metabolic response compared with water snakes. While fasting, pythons cease gastric acid and intestinal base secretion, both of which are stimulated with feeding. In contrast, fasted water snakes secreted gastric acid and intestinal base at rates similar to those of digesting snakes. We observed no difference between fasted and fed individuals for either species in gastric or intestinal transepithelial potential and conductance, with the exception of a significantly greater gastric transepithelial potential for fed pythons at the start of titration. Water snakes experienced no significant change in gastric or intestinal metabolism with feeding. Fed pythons, in contrast, experienced a near-doubling of gastric metabolism and a tripling of intestinal metabolic rate. For fasted individuals, the metabolic rate of the stomach and small intestine was significantly lower for pythons than for water snakes. The fasting downregulation of digestive function for pythons is manifested in a depressed gastric and intestinal metabolism, which selectively serves to reduce basal metabolism and hence promote survival between infrequent meals. By maintaining elevated GI performance between meals, fasted water snakes incur the additional cost of tissue activity, which is expressed in a higher standard metabolic rate.

Key words: digestion, electrophysiology, gastric acid, intestinal base, *Nerodia rhombifer*, postprandial, *Python molurus*, reptile, snake, specific dynamic action, tissue metabolism.

INTRODUCTION

Among snakes there is an adaptive interplay between their feeding habits and the degree to which they regulate digestive performance (Secor and Diamond, 2000; Secor, 2005a). Actively foraging snakes tend to feed relatively frequently in the wild and experience modest changes in intestinal structure and function across feeding and fasting bouts (Secor and Nagy, 1994; Secor and Diamond, 2000; Secor, 2005a). Snakes that employ the sit-and-wait tactic of foraging feed more infrequently, such that meal digestion can be a relatively rare event (Secor and Nagy, 1994; Greene, 1997; Murphy and Henderson, 1997). For these snakes, feeding is accompanied by a very rapid and dramatic upregulation in gastrointestinal (GI) structure and function, which is maintained until digestion is completed (Secor and Diamond, 2000; Ott and Secor, 2007), after which their intestinal epithelium undergoes atrophy and gastric and intestinal function are both severely downregulated (Secor and Diamond, 2000; Secor, 2003; Lignot et al., 2005; Ott and Secor, 2007).

An adaptive explanation for this distinct dichotomy in the regulation of digestive performance among snakes resides in the selective pressure of energy conservation. It is hypothesized that the wide regulation of GI performance is selectively advantageous for infrequently feeding snakes given that a downregulated gut reduces individual energy expenditure during long and predicted episodes of fasting (Secor, 2001; Secor, 2005a). The cumulative energy saved while fasting is expected to exceed the additional energy spent to upregulate the gut with feeding, as well as any extra costs of maintaining the regulatory machinery. For frequently feeding snakes, selection for modest regulation of the gut is favored because this eliminates the frequent cost of upregulation, which may not be balanced or exceeded by the savings of a downregulated gut during their short fasting bouts (Secor, 2001; Secor, 2005a). Models have illustrated that the modest regulation of GI function is favored energetically for snakes that feed at least once every 2 weeks and that the wide regulation of gut performance is energetically advantageous for snakes that feed once every 4 weeks or more (Secor and Diamond, 2000; Secor, 2005a).

In this adaptive scenario, energy conservation as a selective force would be manifested in species differences in whole-animal expenditure during fasting bouts (i.e. standard metabolic rate, SMR). For 20 species of snakes, an allometric comparison of SMR

revealed that infrequently feeding snakes possess SMRs that on average are 50% lower than those of frequently feeding species (Ott and Secor, 2007). The metabolic costs of all digestive tissues, each a function of tissue mass and cellular activities, collectively contributes to an individual's SMR. Hence, a condition of the above evolutionary proposal is that infrequently feeding snakes possess a depressed GI metabolism compared with frequently feeding snakes. As a consequence of the postprandial regulatory response, the gut metabolism of infrequently feeding snakes will increase sharply with feeding as digestive tissues upregulate their performance, whereas the gut metabolism of frequently feeding snakes would not be significantly altered by feeding. The metabolic responses of the GI tract of these snakes to feeding and fasting and the extent to which the dynamics of gut metabolism can be explained by tissue function and ultimately feeding strategy are presently unknown. This information is vital to the construction of an adaptive hypothesis whereby the energy conserved during fasting is a selective incentive to the wide regulation of GI performance among infrequently feeding snakes.

To examine the functional link among metabolism, the postprandial metabolic response and GI function, we compared preprandial and postprandial metabolic rates and gastric and intestinal acid–base secretion, epithelial conductance and oxygen consumption between the frequently feeding diamondback water snakes, *Nerodia rhombifer* (Hallowell), and infrequently feeding Burmese pythons, *Python molurus* (Linnaeus). These snakes exhibit distinctly different feeding habits and regulatory patterns of intestinal performance. The diamondback water snake is an active forager that experiences little change in intestinal morphology and nutrient uptake between fasting and feeding bouts (Cox and Secor, 2010). The Burmese python is a sit-and-wait predator that widely regulates intestinal structure and function with each meal (Secor and Diamond, 1995; Cox and Secor, 2008; Secor, 2008). We set out in this study to: (1) compare the standard metabolic rate and the postprandial metabolic responses of these two species; (2) measure preprandial and postprandial rates of gastric acid and intestinal base secretion; (3) quantify transepithelial potential and conductance of fasted and fed gastric and intestinal tissues; and (4) measure oxygen consumption rates of the stomach and small intestine from fasted and fed animals.

We found pythons to possess a significantly lower SMR and to experience a much larger postprandial metabolic response compared with water snakes. For fasted pythons, the downregulated GI tract was characterized by a lower metabolic rate. Feeding for pythons triggered gastric acid and intestinal base secretion and a matched increase in metabolic rate. In contrast, fasting water snakes possess an active gut with a comparatively elevated metabolism that changed little with feeding.

MATERIALS AND METHODS

Animals and maintenance

The Burmese python, one of the largest species of snake in the world, employs a sit-and-wait foraging tactic that balances reduced foraging costs with a relatively low rate of prey capture (Murphy and Henderson, 1997). Burmese pythons used in this study were purchased as hatchlings (Strictly Reptiles Inc., Hollywood, FL, USA) and housed individually in 20 l plastic containers held within customized racks (Animal Plastics, Johnston, IA, USA). The racks were fitted with a heat cable that provided a front to back temperature gradient of 28–32°C within the containers. Pythons were maintained under a 14 h:10 h L:D photoperiod, fed biweekly on a diet of adult mice or small rats, and provided with water *ad libitum*.

The diamondback water snake is an actively foraging snake that feeds predominately on fish (Mushinsky and Hebrard, 1977; Gibbons and Dorcas, 2004). Field studies have noted the high occurrence of food within their stomachs and laboratory studies have found that their stomachs are cleared 4–5 days after feeding (Mushinsky and Hebrard, 1977; Kofron, 1978; Manjarrez and Macias Garcia, 1991; Aldridge et al., 2003; Cox and Secor, 2010). With a more frequent feeding habit, diamondback water snakes are actively digesting for most of their activity season (Secor and Diamond, 2000; Cox and Secor, 2010). Diamondback water snakes were captured by hand from commercial catfish ponds in Leflore County, MS, USA. At these ponds, watersnakes have continuous access to food (channel catfish, *Ictalurus punctatus*) and were frequently observed feeding. We maintained water snakes in a large tank (3000 l), under a 14 h:10 h L:D photoperiod, at 25–28°C. Water snakes were fed catfish fillets weekly and had continuous access to water.

We use seven pythons (mean mass ± 1 s.e.m., 456±21 g) and eight water snakes (352±9 g) to measure preprandial and postprandial whole-animal metabolic rate. An additional 19 pythons (428±9 g) and six water snakes (391±15 g) were shipped by air from the University of Alabama to the University of Miami Rosenstiel School of Marine and Atmospheric Science to study gastric acid and intestinal base secretion, electrophysiology, and gastric and intestinal metabolic rates. These measurements were performed on pythons following a 30 day fast (N=6) or at 1 (N=3), 2 (N=2), 3 (N=6) or 4 days (N=2) following the consumption of a single rat meal weighing 25.0±0.1% of snake body mass. Mean body mass differed significantly (ANOVA, P<0.021) among the five feeding treatments as pythons at 3 days postfeeding were significantly heavier than fasted snakes. The same set of measurements was performed on water snakes fasted for 15 days (N=3) or at 2 days (N=3) following the consumption of catfish fillet meals that weighed 25.1±0.1% of snake body mass. Mean body mass of water snakes did not differ between the fasted and fed groups.

Snakes were killed by severing their spinal cord immediately posterior to the head and their GI tract was exposed by a mid-ventral incision. All organs were removed and weighed and segments of the anterior stomach and small intestine were immediately placed in aerated ice-cold reptile Ringer's solution (in mmol l^{-1}: 128 NaCl, 20 $NaHCO_3$, 4.7 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4 and 1.2 $MgSO_4$) (Secor et al., 1994) for study of acid/base secretion, electrophysiology and oxygen consumption. Care of and experimentation on snakes were approved by the Institutional Animal Care and Use Committees of the University of Alabama and the University of Miami.

Preprandial and postprandial whole-animal metabolic rate

We quantified preprandial and postprandial metabolic rate (as rate of oxygen consumption, $\dot{V}_{O_2}$), of pythons and water snakes using closed-system respirometry (Secor and Diamond, 1997). Snakes fasted for a minimum of 30 days were placed into individual respirometry chambers constructed from 2.7–4.5 l plastic containers fitted with incurrent and excurrent ports and stopcocks. Respirometry chambers were maintained within a temperature-controlled environmental cabinet at 23°C with air pumped continuously through the incurrent port. For each sampling time point, a 40 ml gas sample was drawn from each chamber through the excurrent port and the chambers were sealed by closing the incurrent and excurrent stopcocks. One to two hours later, the excurrent stopcock was opened and a second 40 ml gas sample was drawn from each chamber. Gas samples were pumped (120 ml min^{-1}) into an O_2

analyzer (S-3A/II, AEI Technologies, Naperville, IL, USA) after passing through a water absorbent (Drierite; W. A. Hammond Drierite Co., Xenia, OH, USA) and CO_2 absorbent column (Ascarite II; Thomas Scientific, Swedesboro, NJ, USA). We calculated $\dot{V}_{O_2}$ ($ml\,h^{-1}$) corrected for standard pressure and temperature as described elsewhere (Vleck, 1987). For each fasted snake we measured $\dot{V}_{O_2}$ twice a day (morning and evening) for 4 consecutive days and assigned the average of the two lowest recorded $\dot{V}_{O_2}$ values as its standard metabolic rate (SMR). Snakes were then removed from their respirometry chambers, given water, and fed either a small rat (pythons) or catfish fillet (water snakes) that weighed approximately 25% (25.2±0.1%) of the snake's body mass. Metabolic measurements were resumed at 12 h intervals (starting at 08:00 h and 20:00 h) for 4 days and continued thereafter at 24 h intervals (starting at 08:00 h) for 8–11 additional days. On the fifth and tenth day after feeding, after morning metabolic measurements, snakes were removed from their chambers, provided with water and then returned to their chambers.

Gastric and intestinal acid–base secretion and electrophysiology

We quantified gastric acid and intestinal base secretion while simultaneously measuring electrophysiological parameters for fasted and fed water snakes and pythons using a pair of Ussing chamber systems (Physiological Instruments, San Diego, CA, USA) as described previously (Grosell and Genz, 2006). In brief, segments of the anterior stomach and small intestine were mounted onto tissue holders (model P2413, Physiological Instruments) which exposed 0.71 cm^2 of each side of the tissue. The tissue holders were positioned between the two half-chambers (model P2400; Physiological Instruments) of each Ussing system. Each half-chamber contained 1.6 ml of pre-gassed reptile Ringer's solution (Secor et al., 1994) with 5 $mmol\,l^{-1}$ glucose added to the serosal side, and was continuously aerated (100% O_2 mucosal side: 95% O_2, 5% CO_2 serosal side), and maintained at a constant temperature of 23°C.

Each Ussing chamber was set up in combination with an automated pH-stat titration system (model TIM 854 or 856, Radiometer, Copenhagen, Denmark) allowing for quantification of acid and base secretion from the gastric and intestinal epithelia, respectively. A pH electrode (model PHC4000.8, Radiometer) and microburette tip were submersed in the luminal half-chamber of each system. For the gastric epithelia, pH-stat titration commenced once gastric mucosal saline had dropped to a pH of 4.00; thereafter base titrant (0.005 $mol\,l^{-1}$ NaOH) was added *via* the microburette to maintain the mucosal saline at pH 4.00. Similarly, for the intestinal epithelia, pH-stat titration began once intestinal mucosal saline had increased to pH 7.80, after which acid titrant (0.005 $mol\,l^{-1}$ HCl) was added to maintain the mucosal saline at pH 7.80 (Taylor and Grosell, 2009).

For each system, pH and titrant volumes were continuously recorded (Titramaster software, version 5.1) allowing for calculation of rates of gastric acid and intestinal base secretion. We assumed that the titratable acid and base secreted are HCl and HCO_3^-, respectively (Grosell and Genz, 2006). For gastric and intestinal tissues we report on the following parameters: (1) initial pH, averaged over the first 10 min of measurements; (2) rate of solution acidification or alkalinization, averaged over the first 10 min of measurements; (3) time to titration, the time it took for the gastric or intestinal preparation to reach a pH of 4.00 or 7.80, respectively; and (4) rate of titration, averaged over the first 10 min of titration for those preparations that reached titratable pH.

Additionally, for each Ussing chamber system, current and voltage electrodes connected to amplifiers (model VCC600; Physiological Instruments) recorded the transepithelial potential (TEP) differences with a mucosal reference (0 mV), under current-clamp conditions at 0 μA. Epithelial conductance was quantified by generating 10 μA pulses of 3 s duration from the mucosal to the serosal side of the tissues at 60 s intervals. For each acid–base system, the automated pH-stat titration system was grounded to the amplifier to allow for continual current pulsing without interfering with pH measurements. Current and TEP measurements were continuously recorded by a computer-linked data-acquisition system (MP100, Biopac Systems, Santa Barbara, CA, USA) and Acqknowledge software (Biopac Systems).

After ensuring stable electrophysiological parameters for each epithelial preparation, TEP and conductance were analyzed in parallel with the acid–base measurements. For the two (out of six) python gastric samples that did not reach titration pH at day 3, TEP and conductance at titration were recorded at 29.3 min (the mean time to titration for the other four samples). For all fasted and day 4 samples, gastric TEP and conductance at titration were noted at 37.4 min, the overall mean time to titration for all samples that resulted in base titration.

Gastric and intestinal O_2 consumption

We measured rates of oxygen consumption ($\dot{V}_{O_2}$ in $\mu mol\,h^{-1}$) of the mucosal and serosal surfaces of gastric and intestinal tissues using custom-designed glass Ussing chambers (model CH10500, Loligo Systems, Tjele, Denmark) as described previously (Taylor and Grosell, 2009). In brief, segments of the anterior stomach and small intestine were placed in Teflon holders constructed to expose a circular (0.87 cm^2) region of the mucosa and serosa. Each half-chamber (2.95 ml) was filled with aerated (100% air) reptile Ringer's solution (5 $mmol\,l^{-1}$ glucose added to the serosal side), which was continually mixed *via* micro-magnetic stir bars. Fiber-optic oxygen sensors and a light source (Loligo Systems) positioned within each half-chamber allowed us to monitor the change in oxygen concentration independently for the mucosal and serosal surfaces. Oxygen sensors were connected to single-channel oxygen meters (Fibrox 3, Loligo Systems). Parallel measurements of oxygen saturation for the mucosal and serosal half-chamber were recorded using Oxy-View Software (www.oxyview.com).

We calibrated O_2 sensors daily by gassing the reptile Ringer's solution within each half-chamber with N_2 to zero the system, and then gassing the Ringer's solution with 100% air to set the maximum percentage oxygen saturation. We quantified $\dot{V}_{O_2}$ in each half-chamber over the range of 100–90% oxygen saturation, as employed previously for the gulf toadfish *Opsanus beta* (Taylor and Grosell, 2009). We combined measurements from mucosal and serosal half-chambers to quantify whole-tissue segment $\dot{V}_{O_2}$, which we divided by tissue mass for mass-specific rates ($ml\,O_2\,g^{-1}\,h^{-1}$). Whole-organ (stomach and small intestine) metabolic rate was calculated as the product of organ mass and mass-specific $\dot{V}_{O_2}$.

Statistical analyses

To compare SMRs of water snakes (mean mass 352 g) and pythons (mean mass 456 g) that differed significantly in mean body mass, we allometrically adjusted individual SMR to the mean body mass of the other species using mass exponents of 0.68 and 0.75. Hence, the SMR of each water snake was adjusted to a body mass of 456 g assuming allometric exponents of 0.68 and 0.75. Likewise for each python, the same adjustments to SMR were made to a common

body mass of 352 g. Allometrically adjusted SMRs were compared interspecifically by analysis of variance (ANOVA).

For postprandial metabolic trials, we used repeated-measures ANOVA to test for significant effects of time (before and after feeding) on $\dot{V}_{O_2}$. Each ANOVA was accompanied by a *post hoc* pairwise mean comparison to identify when $\dot{V}_{O_2}$ increased significantly after feeding and when postprandial $\dot{V}_{O_2}$ did not significantly differ from SMR. To investigate the effects of feeding on tissue and organ mass, acid–base secretion, electrophysiology and metabolic rate for each species, we used ANOVA for pH, rates of acid production, electrophysiology and mass-specific $\dot{V}_{O_2}$, and analysis of covariance (ANCOVA with body mass as a covariate) for organ masses and integrated organ metabolism. ANOVA and ANCOVA were used for interspecific comparisons of measured or calculated parameters. For pythons, significant ANOVA and ANCOVA were followed by pairwise mean comparisons to identify differences between feeding treatments. We report the results of the ANOVA and ANCOVA tests in terms of their *P*-values and note the *P*-values of significant pairwise mean comparisons. The level of statistical significance for this study was set at $P<0.05$. Mean values are reported ±1 s.e.m.

RESULTS

Whole-animal metabolic rate

Regardless of the allometric correction (0.68 or 0.75) to a common mean body mass (352 or 456 g), SMR (23°C) of adult diamondback water snakes was on average 40% greater (all $P<0.003$) than that of juvenile Burmese pythons (Fig. 1). Both snake species experienced significant postprandial increases in $\dot{V}_{O_2}$ within 12 h of feeding (Fig. 2). Postprandial $\dot{V}_{O_2}$ of water snakes peaked with a plateau between 2.5 and 5 days postfeeding at 4.6- to 4.8-fold SMR. Python postprandial $\dot{V}_{O_2}$ peaked at 3 days postfeeding at 8.9-fold SMR. Although the postprandial peak $\dot{V}_{O_2}$ of pythons was on average 17% greater (scaled to common body mass) than peak $\dot{V}_{O_2}$ of water snakes, the difference was not significant. Both species experienced a return of $\dot{V}_{O_2}$ to rates not significantly different from SMR by day 10 of digestion. Over these 10 days, the cumulative energy expended on digestion and assimilation (specific dynamic action, SDA) was significantly ($P<0.035$) greater for pythons (376±6 kJ kg^{-1}) than for water snakes (323±21 kJ kg^{-1}). In contrast, the SDA coefficient (SDA as a percentage of meal energy) was significantly ($P<0.006$) greater for water snakes (28.0±1.8%) than for pythons (21.1±0.3%).

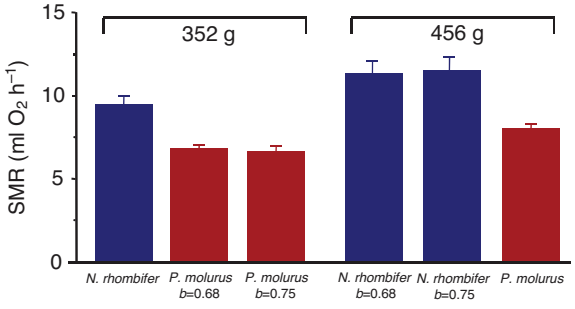

Fig. 1. Standard metabolic rate (SMR) of diamondback water snakes (*Nerodia rhombifer*) and Burmese pythons (*Python molurus*). Species comparisons are illustrated by adjusting SMR to common body masses (352 and 456 g) using traditional allometric scaling exponents of 0.68 and 0.75. Error bars indicate +1 s.e.m. Regardless of body mass and scaling exponent, *N. rhombifer* exhibit significantly greater SMR than *P. molurus*.

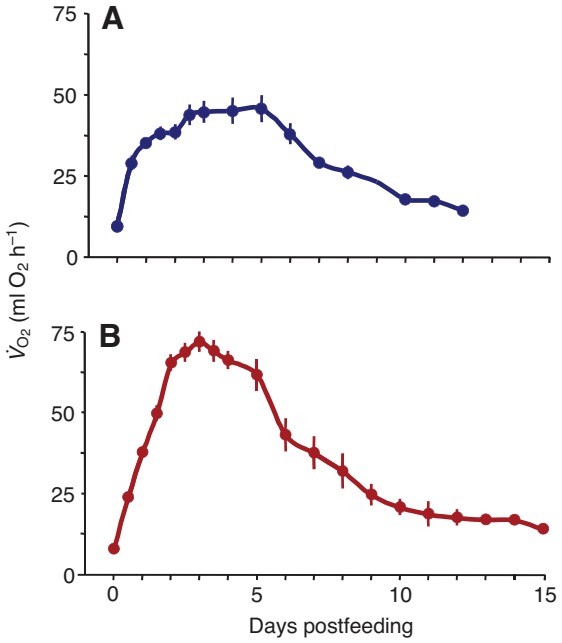

Fig. 2. Mean (±1 s.e.m.) rates of oxygen consumption prior to (day 0) and up to 15 days following the consumption of (A) catfish fillet meals for diamondback water snakes (*N. rhombifer*, *N*=8) and (B) rodent meals for Burmese pythons (*P. molurus*, *N*=7). Meals were equal in mass to 25% of snake body mass, and measurements were conducted at 23°C.

Stomach and intestinal contents and organ masses

Following 2 days of digestion, stomach contents for water snakes had decreased by 69±6%. After 1, 2, 3 and 4 days of digestion, stomach contents for pythons had been reduced by 15±3%, 40±9%, 72±3% and 98±1%, respectively. After 2 days, small intestinal contents for water snakes averaged 2.5±0.5 g and peaked at 9.5±0.8 g for pythons. We observed no significant effects of feeding on the wet masses of the esophagus, lungs, heart, liver, stomach, empty gall bladder, pancreas, spleen, small intestine, large intestine or kidneys for water snakes (Table 1). The only significant ($P<0.04$) change was a 52% decrease in the wet mass of the full gall bladder due to the postprandial release of bile into the small intestine. In contrast, pythons experienced significant (all $P<0.05$) postprandial increases in the mass of four organs; liver (by 93%), pancreas (85%), small intestine (61%) and kidneys (18%) (Table 1). Like the water snake, the python's gall bladder released bile with feeding, decreasing its full mass by 53%.

Gastric and intestinal acid–base secretion

The initial pH of luminal solutions employed for gastric tissue preparations did not differ between fasted and fed water snakes (Table 2). For both fasted and fed snakes, gastric tissues secreted acid such that solution pH decreased at similar rates (0.02–0.04 pH units min^{-1}). For all six water snakes (fasted and fed), mucosal solution reached pH 4.00 within 25–90 min (Table 2, Fig. 3A). Base titration then commenced at a rate of 0.53–1.15 µmol cm^{-2} h^{-1} (Table 2, Fig. 3A). For water snakes, fed individuals averaged a greater rate of gastric acid production (faster drop in pH, earlier titration, and higher titration rates) than fasted individuals, though these differences were not statistically significant (Table 2).

In contrast, initial luminal gastric solution pH differed significantly among fasted and fed pythons (Table 2). For snakes 1, 2 or 3 days

Table 1. Body mass (g) and wet mass (g) of organs of fasted and fed (2 days post-feeding, 2 DPF) diamondback water snakes (*Nerodia rhombifer*) and fasted and fed (1, 2, 3 and 4 DPF) Burmese pythons (*Python molurus*)

	N. rhombifer			*P. molurus*					
Mass (g)	Fasted (*N*=3)	2 DPF (*N*=3)	*P*	Fasted (*N*=6)	1 DPF (*N*=3)	2 DPF (*N*=2)	3 DPF (*N*=6)	4 DPF (*N*=2)	*P*
Body	412±25	377±3	0.235	460±19^{a}	478±10^{a,b}	486±13^{a,b}	531±8^{b}	496±12^{a,b}	0.021
Esophagus	3.49±0.21	4.80±0.86	0.358	7.50±0.41	7.16±0.44	6.98±1.04	6.92±0.37	6.83±0.83	0.890
Lungs	3.80±0.74	2.98±0.34	0.902	7.12±1.36	5.61±1.54	4.29±0.50	4.86±0.69	9.04±1.10	0.264
Heart	1.28±0.33	0.98±0.03	0.978	1.11±0.07	1.31±0.21	1.05±0.11	1.28±0.06	1.26±0.12	0.349
Liver	8.67±0.91	10.71±0.50	0.172	5.94±0.60^{a}	9.58±0.65^{b}	9.85±0.09^{b}	11.15±0.41^{b}	12.37±1.06^{b}	0.0006
Stomach	3.78±0.23	4.16±0.10	0.209	6.82±0.41	8.28±0.48	8.12±0.51	8.36±0.29	8.83±0.29	0.127
Gall bladder (full)	1.06±0.18	0.46±0.07	0.037	1.82±0.21^{b}	1.72±0.06^{a,b}	0.95±0.03^{a,b}	0.94±0.07^{a}	0.93±0.12^{a,b}	0.014
Spleen	0.10±0.03	0.10±0.01	0.542	0.09±0.01	0.14±0.02	0.12±0.01	0.11±0.01	0.14±0.01	0.084
Pancreas	0.49±0.07	0.62±0.05	0.180	0.39±0.02^{a}	0.61±0.09^{b}	0.62±0.03^{b}	0.62±0.03^{b}	0.79±0.02^{b}	0.0013
Small intestine	6.76±0.93	10.36±0.52	0.075	6.26±0.18^{a}	9.28±0.95^{b}	10.66±1.96^{b}	8.92±0.44^{b}	9.88±0.21^{b}	0.0027
Cecum				0.40±0.13	0.49±0.08	0.35±0.05	0.30±0.03	0.39±0.02	0.936
Large intestine	1.74±0.28	1.90±0.35	0.598	4.67±0.48	4.96±0.26	5.17±0.71	5.10±0.38	6.01±0.11	0.565
Kidneys	7.44±0.90	8.33±0.77	0.358	2.70±0.26^{a}	3.10±0.25^{a,b}	3.26±0.15^{a,b}	3.65±0.12^{b}	3.30±0.17^{a,b}	0.047

Values are presented as means ± 1 s.e.m. For body and organ masses, different superscript letters denote significant (P<0.05) differences between means, as determined from *post hoc* pairwise comparisons.

into digestion, the initial pH of the gastric solution averaged more than half of a pH unit less than that for stomach preparations from fasted snakes. Neither fasted snakes nor snakes 4 days into digestion exhibited gastric acid release (Table 2, Fig. 3C). Within minutes following introduction into the chambers, gastric tissues sampled at 1, 2 and 3 days postfeeding began to acidify the mucosal solution (Fig. 3C). A mucosal solution pH of 4.0 was reached (usually within 45 min) by all 1 day, all 2 day, and four of the six 3 day pythons. For these nine snakes, acid secretion (not differing among 1, 2 and 3 day snakes) averaged 0.93±0.07 μmol cm^{-2} h^{-1}.

Initial pH and rate of alkalization of the intestinal mucosal solution did not differ between fasted and fed water snakes (Table 2, Fig. 3B). After 45 min, mucosal solutions of both fasted and fed tissues experienced a significant increase in pH, with a significantly greater pH reached for tissues from fed snakes (Table 2). In spite of alkalization of the mucosal solution, solution pH never reached 7.80 for either fasted or fed water snakes; hence, we were unable to quantify their intestinal base secretion rates.

For pythons, initial intestinal pH differed significantly (P=0.0012) among the five feeding treatments (Table 2). Fed snakes possessed

Table 2. pH-stat measurements in gastric and intestinal epithelia from fasted and fed (2 DPF) diamondback water snakes (*Nerodia rhombifer*) and fasted and fed (1, 2, 3 and 4 DPF) Burmese pythons (*Python molurus*)

	N. rhombifer			*P. molurus*					
	Fasted (*N*=3)	2 DPF (*N*=3)	*P*	Fasted (*N*=6)	1 DPF (*N*=3)	2 DPF (*N*=2)	3 DPF (*N*=6)	4 DPF (*N*=2)	*P*
Gastric									
Initial pH	5.45±0.14	5.33±0.28	0.725	5.63±0.10	4.95±0.28	4.93±0.75	5.03±0.16	5.45±0.04	0.109
Initial acidification (pH units min^{-1})	–0.025±0.016	–0.038±0.013	0.532	0.006±0.005^{a,*}	–0.057±0.003^{b}	–0.024±0.009^{a,b}	–0.036±0.011^{b}	0.016±0.005^{a}	0.0012
Titration (*N*)	3	3		0	3	2	4	0	
Time to titration (min)	69.4±10.4	49.6±14.9	0.335		30.1±10.0	64.6±56.7	29.3±4.4		0.933
Titration rate (μmol cm^{-2} h^{-1})	0.59±0.05	0.95±0.18	0.121		0.95±0.11	0.96±0.30	0.92±0.07		0.503
Intestinal									
Initial pH	6.08±0.11	6.30±0.0.05	0.153	6.06±0.04^{a}	6.18±0.03^{a,b}	6.73±0.27^{c}	6.32±0.06^{b}	6.12±0.09^{a,b}	0.0012
Initial alkalinization (pH units min^{-1})	0.027±0.003	0.029±0.004	0.693	0.016±0.002^{a,*}	0.035±0.002^{b}	0.021±0.013^{a,b}	0.033±0.002^{b}	0.031±0.003^{b}	0.0046
pH after 45 min	6.49±0.09	6.74±0.01	0.041	6.36±0.03^{a}	6.78±0.03^{b,c}	7.08±0.12^{c}	6.90±0.07^{c}	6.60±0.08^{b}	<0.0001
Alkalinization after 45 min (pH units min^{-1})	0.005±0.001	0.005±0.001	0.519	0.004±0.001^{a}	0.035±0.002^{c,†}	0.004±0.001^{a,b}	0.007±0.001^{b}	0.006±0.001^{a,b}	<0.0001
Titration (*N*)	0	0		0	0	0	1	0	
Time to titration (min)							71.4		
Titration rate (μmol cm^{-2} h^{-1})							0.61		

Variables are explained in the text and values are presented as means ± 1 s.e.m. Measurements are reported at experiment onset (initial) and after 45 minutes for intestinal samples. For *P. molurus*, superscript letters that differ denote significant (P<0.05) differences between means as determined from *post hoc* pairwise comparisons. *Significant difference between fasted *N. rhombifer* and fasted *P. molurus*. †Significant difference between fed (2 DPF) *N. rhombifer* and fed (1–4 DPF) *P. molurus*.

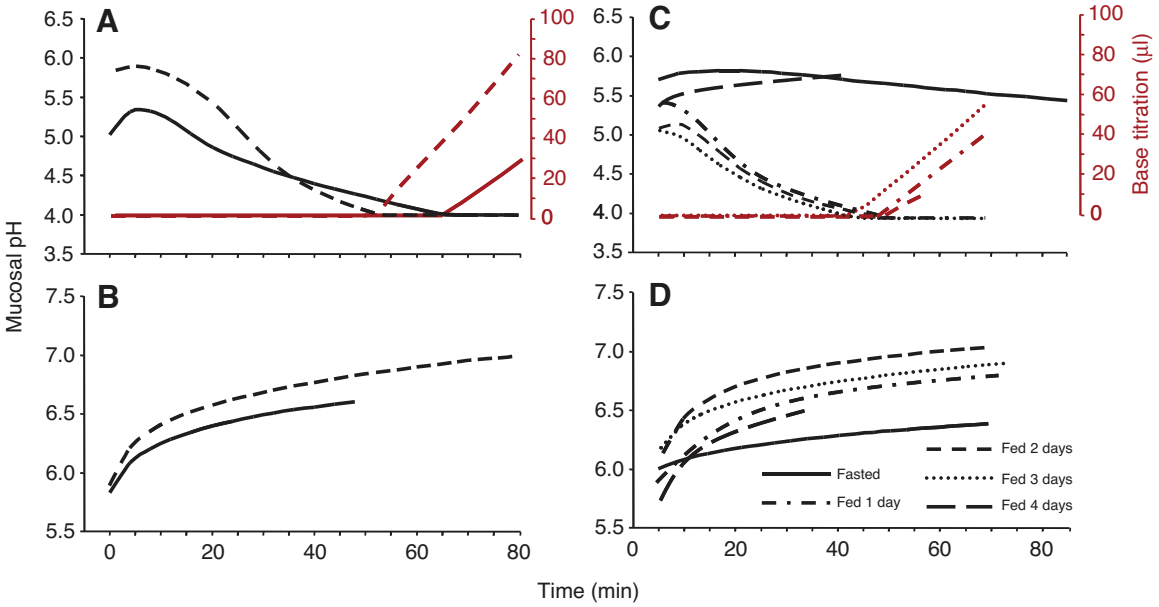

Fig. 3. pH traces of representative segments of stomach (A,C) and small intestine (B,D) for fasted and fed diamondback water snakes (*N. rhombifer*; A,B) and Burmese pythons (*P. molurus*; C,D). Gastric acid secretion rates for fasted and fed *N. rhombifer* and fed *P. molurus* are indicated by volume of base titration along the right *y*-axis for A and C.

intestinal mucosal solution pH values that averaged as much as 0.67 pH units greater than for fasted snakes immediately after being mounted in the Ussing chambers (Table 2, Fig. 2D). The initial rate of alkalinization of the intestinal solution for fed pythons was twice that of fasted individuals (Table 2). In only one instance did a fed python intestine (3 day) alkalinize the mucosal solution to pH 7.80 (after more than 70 min). Thereafter, base secretion proceeded at $0.61\,\mu mol\,cm^{-2}\,h^{-1}$.

When we compare results for water snakes with those for pythons, initial rates of gastric solution acidification and intestinal solution alkalinization were significantly (both $P<0.05$) greater in fasted water snakes than in fasted pythons (Table 2). With feeding, however, initial rates of gastric solution acidification, the time to base titration, and rates of titration were similar between the two species. Likewise, the initial rate of alkalinization of the intestinal solution did not differ between water snakes and pythons after feeding (Table 2). As noted, only one fed python intestine increased solution pH to 7.80, whereas none of the fed water snake samples did so.

Gastric and intestinal electrophysiology

Because of high inter-individual variation, we detected no significant postprandial change in gastric TEP and conductance for water snakes

Table 3. Electrophysiological measurements of gastric and intestinal epithelia from fasted and fed (2 DPF) diamondback water snakes (*Nerodia rhombifer*) and fasted and fed (1, 2, 3 and 4 DPF) Burmese pythons (*Python molurus*)

	N. rhombifer			*P. molurus*					
	Fasted (*N*=3)	2 DPF (*N*=3)	*P*	Fasted (*N*=6)	1 DPF (*N*=3)	2 DPF (*N*=2)	3 DPF (*N*=6)	4 DPF (*N*=2)	*P*
Gastric									
Initial TEP (mV)	0.90±1.52	0.18±1.16	0.724	3.05±0.89	5.04±0.46†	1.52±0.57	3.31±0.59†	1.28±0.48	0.117
TEP at titration (mV)	1.29±4.05	–0.52±0.73	0.683	1.47±0.83^{a}	5.67±1.31^{c,†}	2.91±1.19^{a,b,c}	4.37±0.94^{b,c,†}	1.26±1.10^{a,b}	0.050
Initial conductance ($mSi\,cm^{-2}$)	7.63±0.58	7.18±0.09	0.490	5.38±0.71	5.45±0.36†	6.52±0.18†	5.71±0.46†	6.54±0.06†	0.703
Conductance at titration ($mSi\,cm^{-2}$)	7.27±0.65	8.07±0.08	0.287	5.18±0.72	6.54±0.40†	6.57±0.20†	6.59±0.35†	6.46±0.06†	0.301
Intestinal									
Initial TEP (mV)	6.16±1.86	2.12±3.75	0.389	0.33±1.13*	0.13±0.24	–1.43±0.09	1.59±0.53	2.96±1.18	0.187
TEP after 45 min (mV)	0.80±1.69	–1.12±2.38	0.546	0.23±1.71	0.03±0.14	–1.60‡	1.56±0.63	3.32±1.01	0.535
Initial conductance ($mSi\,cm^{-2}$)	7.330±0.48	7.52±0.26	0.736	5.68±0.51	7.66±0.19	5.63±1.02	7.32±0.77	6.55±0.24	0.209
Conductance after 45 min ($mSi\,cm^{-2}$)	8.38±0.32	7.64±0.61	0.348	6.12±0.67	8.12±0.05	6.85‡	7.44±0.83	7.16±0.26	0.483

Variables are explained in the text and values are presented as means ± 1 s.e.m. TEP values are reported with a mucosal reference of 0 mV. Measurements are reported at experiment onset (initial) and after 45 min for intestinal samples. For *P. molurus*, superscript letters that differ denote significant ($P<0.05$) differences between means as determined from *post hoc* pairwise comparisons. *Significant difference between fasted *N. rhombifer* and fasted *P. molurus*. †Significant difference between fed (2 DPF) *N. rhombifer* and fed (1–4 DPF) *P. molurus*. ‡Sample size of 1.

Table 4. Oxygen consumption rates of the mucosal and serosal sides and entire gastric and intestinal tissue (0.87 cm^2) from fasted and fed (2 DPF) diamondback water snakes (*Nerodia rhombifer*) and fasted and fed (1, 2, 3 and 4 DPF) Burmese pythons (*Python molurus*)

	N. rhombifer			*P. molurus*					
	Fasted (*N*=3)	2DPF (*N*=3)	*P*	Fasted (*N*=6)	1 DPF (*N*=3)	2 DPF (*N*=2)	3 DPF (*N*=6)	4 DPF (*N*=2)	*P*
Gastric									
Mucosa (μmol O_2 cm^{-2} h^{-1})	0.313±0.036	0.297±0.049	0.804	0.183±0.022^{a,*}	0.241±0.014^{a}	0.245±0.038^{a}	0.349±0.037^{b}	0.276±0.062^{a}	0.016
Serosa (μmol O_2 cm^{-2} h^{-1})	0.209±0.010	0.218±0.025	0.757	0.160±0.024	0.147±0.005	0.190±0.017	0.221±0.051	0.166±0.014	0.670
Total (ml O_2 g^{-1} h^{-1})	0.082±0.001	0.117±0.021	0.169	0.024±0.003^{a,*}	0.054±0.013^{b,†}	0.053±0.012^{b,†}	0.056±0.008^{b,†}	0.043±0.004^{a,b,†}	0.025
Intestinal									
Mucosa (μmol O_2 cm^{-2} h^{-1})	0.301±0.038	0.402±0.047	0.167	0.269±0.011^{a}	0.517±0.072^{b}	0.734±0.059^{b,c,†}	0.767±0.073^{c,†}	0.507±0.128^{b}	0.0002
Serosa (μmol O_2 cm^{-2} h^{-1})	0.195±0.012	0.200±0.011	0.789	0.161±0.015^{a}	0.382±0.034^{c,†}	0.228±0.028^{a,b}	0.545±0.031^{d,†}	0.348±0.079^{b,c,†}	<0.0001
Total (ml O_2 g^{-1} h^{-1})	0.102±0.007	0.067±0.002	0.098	0.047±0.005^{a,*}	0.119±0.016^{b,c}	0.118±0.002^{b,c}	0.153±0.013^{c,†}	0.081±0.009^{a,b}	<0.0001

Values are presented as means ± 1 s.e.m. For *P. molurus*, different superscript letters denote significant (P<0.05) differences between means as determined from *post hoc* pairwise comparisons. *Significant difference between fasted *N. rhombifer* and fasted *P. molurus*. †Significant difference between fed (2 DPF) *N. rhombifer* and fed (1–4 DPF) *P. molurus*.

(Table 3). Similarly, intestinal tissue TEP and conductance did not differ between fasted and fed water snakes, either initially or after 45 min (Table 3). Likewise, initial gastric TEP and conductance did not significantly vary among fasted and fed pythons (Table 3). However, with titration of the gastric saline, TEP of the gastric tissue was significantly greater for fed (1 and 3 days) than for fasted pythons (Table 3). Gastric conductance, in contrast, did not vary among fasted and fed pythons following the start of titration. Intestinal TEP and conductance did not significantly vary among fasted and fed groups of pythons (Table 3).

Between fasted water snakes and pythons, there was no significant difference in gastric TEP or gastric conductance (initial or at titration) (Table 3). Among fed snakes, pythons tended to exhibit significantly greater gastric TEP and lower gastric conductance, both initially and during titration, than water snakes (Table 3). For the small intestine, initial intestinal TEP was significantly greater for fasted water snakes than for fasted pythons (Table 3). For other electrophysiology measurements of the small intestine, there was no significant difference between water snakes and pythons, either fasted or fed (Table 3).

Gastric and intestinal metabolism

For fasted and fed water snakes, $\dot{V}_{O_2}$ did not significantly differ between the mucosal and serosal sides of gastric tissues (Table 4). For either side of the gastric tissue, $\dot{V}_{O_2}$ did not significantly change with feeding (Table 4). While feeding did generate a 50% increase in gastric tissue $\dot{V}_{O_2}$ (summed mucosal and serosal $\dot{V}_{O_2}$), the change was not statistically significant (P>0.17). When integrating gastric tissue metabolism (ml O_2 g^{-1} h^{-1}) with stomach mass (g), we found that water snakes did experience a significant (P<0.05) postprandial increase (66%) in total gastric $\dot{V}_{O_2}$ (Fig. 4A)

For fasted pythons likewise, there was no significant difference in $\dot{V}_{O_2}$ between the mucosal and serosal sides of the gastric tissues (Table 4). With feeding, mucosal $\dot{V}_{O_2}$ was significantly (both P<0.017) greater than serosal $\dot{V}_{O_2}$ at 1 and 3 days (Table 4). Among fasted and fed treatments, gastric mucosal $\dot{V}_{O_2}$ was significantly (P<0.017) greater at day 3 of digestion compared to fasted snakes (Table 4). We observed no significant variation among fasted and fed sampling times in serosal $\dot{V}_{O_2}$ (Table 4). Gastric tissue $\dot{V}_{O_2}$ (summed mucosa and serosa) differed significantly (P<0.025) among sampling times as rates were elevated at 1, 2 and 3 days (Table 4). Combining tissue metabolic rates and stomach masses, pythons experienced a significant postprandial (1, 2 and 3 days) increase (by as much as 175%) in total stomach metabolism (Fig. 4A).

For fasted water snakes, $\dot{V}_{O_2}$ did not differ between the mucosal and serosal sides of the small intestine (Table 4). During digestion, intestinal mucosal $\dot{V}_{O_2}$ increased (P<0.031) to double that of serosal $\dot{V}_{O_2}$ (Table 4). However, when combining the mucosal and serosal values, intestinal $\dot{V}_{O_2}$ did not significantly increase with feeding for

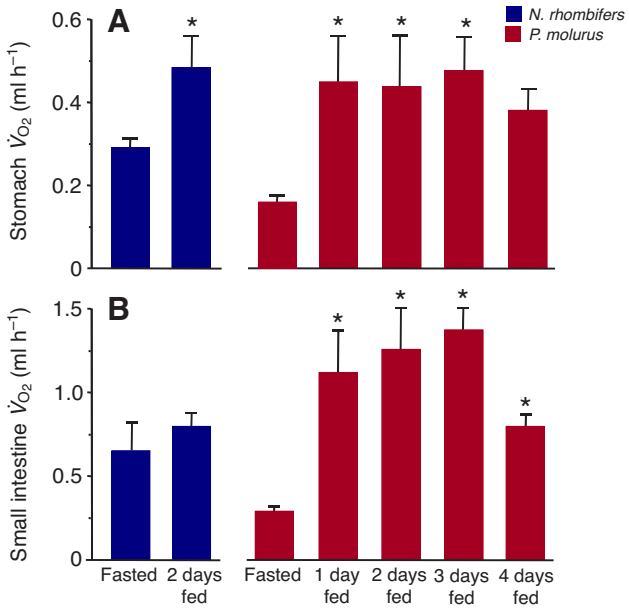

Fig. 4. Calculated oxygen consumption rates for complete intact stomach (A) and small intestine (B) for fasted and fed diamondback water snakes (*N. rhombifer*) and Burmese pythons (*P. molurus*). Note that with feeding, stomach $\dot{V}_{O_2}$ increased significantly (*P<0.05 in pairwise comparisons of fasted $\dot{V}_{O_2}$) for water snakes and pythons and that small intestinal $\dot{V}_{O_2}$ had significantly increased at 1, 2 and 3 days postfeeding for the python.

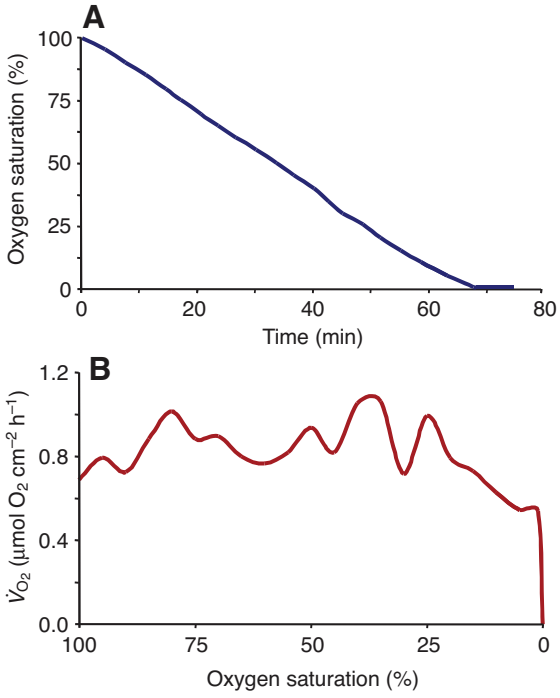

Fig. 5. (A) The decline in oxygen saturation as a function of experimental time for the Ringer's solution in contact with the mucosa of a small intestinal segment from a fed (3 days) Burmese python (*Python molurus*). (B) Oxygen consumption rates of the mucosa of the same intestinal segment as a function of oxygen saturation of the Ringer's solution. Note that even with the severe depletion of oxygen in the surrounding Ringer's solution, the python's intestinal mucosa maintains a constant rate of oxygen consumption until nearly anoxic conditions are reached.

water snakes. Likewise, total intestinal $\dot{V}_{O_2}$ did not significantly differ between fasted and fed water snakes (Fig. 4B).

For fasted pythons, the mucosal side of the small intestine exhibited a significantly (P<0.020) greater $\dot{V}_{O_2}$ compared with the serosal side (Table 4). For the four postprandial time periods, mucosal $\dot{V}_{O_2}$ averaged higher (by 35–220%) than serosal $\dot{V}_{O_2}$, though only significantly (both P<0.05) so at days 2 and 3. Small intestinal mucosal and serosal $\dot{V}_{O_2}$ varied significantly (both P<0.0002) among fasted and fed tissues, each increasing within 24 h after feeding, and again by day 3 of digestion. Hence, intestinal $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) increased with feeding to peak at three times fasting levels at day 3 (Table 4). Combined with the postprandial increase in small intestinal mass, pythons experience an estimated 4.6-fold increase in intestinal metabolism after feeding (Fig. 4B).

For both the gastric and intestinal tissues, mucosal and serosal $\dot{V}_{O_2}$ averaged less for fasted pythons compared to fasted water snakes (significantly so for the gastric mucosa) (Table 4). Mass-specific $\dot{V}_{O_2}$ of gastric and small intestinal segments from fasted pythons averaged only 29% and 46%, respectively, of the $\dot{V}_{O_2}$ of these tissues from fasted water snakes (Table 4). During digestion, following the postprandial increase in gastric $\dot{V}_{O_2}$ for pythons, gastric mucosal and serosal $\dot{V}_{O_2}$ did not differ between water snakes and pythons. Even though pythons experienced a postprandial increase in gastric $\dot{V}_{O_2}$, whereas water snakes did not, water snakes still exhibited a greater gastric metabolism than pythons during meal digestion. In contrast, small intestinal $\dot{V}_{O_2}$ of digesting pythons at day 3 was significantly greater than that of digesting water snakes (Table 4).

DISCUSSION

For the frequently feeding diamondback water snake and the infrequently feeding Burmese python, the metabolic and functional responses of their digestive tracts to feeding and fasting are seemingly integrated into their feeding habits. The water snake maintains intestinal structure and function throughout fasting episodes and hence experiences a modest change in gut metabolism with feeding. In contrast, the python downregulates GI form and function upon the completion of digestion and subsequently has to rapidly upregulate gut performance with feeding. Gastric and intestinal metabolic rates follow suit, depressed during fasting and significantly elevated with feeding. In the following we will examine for these two snakes the interplay between their feeding habits and the metabolic and functional responses of their stomach and small intestine.

Preprandial and postprandial metabolism

Allometric corrections of SMR to a common body mass (352 or 456 g) using scaling exponents of 0.68 and 0.75 generated the same conclusion; diamondback water snakes possess a significantly greater SMR than Burmese pythons at 23°C. An allometric plot of SMR measured at 30°C for seven frequently feeding snakes and 11 infrequently feeding species, including both the diamondback water snake and Burmese python, illustrates a similar finding. Across a 10-fold range in body mass, frequently feeding snakes exhibited SMRs that were 50–70% greater than those of infrequently feeding species (Ott and Secor, 2007). One explanation is that fasted frequently feeding snakes tend to possess relatively larger hearts, livers, pancreases, intestines and kidneys, organs with higher mass-specific metabolic rates, compared with fasted infrequently feeding species (Secor and Diamond, 2000; Ott and Secor, 2007). A caveat to these comparisons is that the infrequent feeders include pythons (Pythonidae), boas (Boidae) and a rattlesnake (Viperidae), and all frequent feeding species are of the family Colubridae. The extent to which phylogeny and feeding habits are each responsible for differences in SMR and organ masses is yet to be resolved.

Both water snakes and pythons experience characteristic postprandial metabolic profiles; an immediate rapid increase in $\dot{V}_{O_2}$ that upon peaking declines more slowly to prefeeding levels (Fig. 2). Similar postprandial profiles have been described for other snake species and a diversity of vertebrate taxa (reviewed in Secor, 2009). Profiles previously documented for diamondback water snakes and Burmese pythons with similar meal sizes share the same general shape to those of this study, though differ in being shorter in duration (Secor and Diamond, 1997; Overgaard et al., 2002; Cox and Secor, 2010). Those studies were conducted at 30°C and $\dot{V}_{O_2}$ of water snakes and pythons returned to prefeeding values within 7 and 8 days, respectively, compared with 10 days in this study, which was conducted at 23°C (Cox and Secor, 2010; Secor and Diamond, 1997). The lower experimental temperature of this study resulted in a reciprocal increase in the duration of digestion and hence a longer metabolic response (Wang et al., 2003; Secor, 2009).

Beginning with a lower SMR and attaining a higher postprandial $\dot{V}_{O_2}$ and hence a greater metabolic scope, pythons of this study expended more energy than water snakes in digesting their meals. This fits the general pattern that when meal type (rodents), meal size (25% of body mass) and body temperature (30°C) are all controlled for, infrequently feeding species experience a significantly greater SDA compared with frequently feeding snakes (Cox and Secor, 2010; Ott and Secor, 2007; Secor and Diamond, 2000). An added explanation stems from their different meal types; intact young rats for pythons and catfish fillets for water snakes. Intuitively, more

effort is expended in breaking down an intact rat than a skinless, boneless catfish fillet. For the Burmese python, the SDA generated from the digestion of a piece of raw beef (no skin or bones) was 9% lower than the SDA from the digestion of an intact rat (Secor, 2003). Whereas SDA coefficients (SDA as a percentage of meal energy) tend to be greater for infrequently feeding snakes (range 15.1–33.0) compared with frequent feeders (range 13.0–16.9), when controlling for meal type the water snakes of this study possessed larger SDA coefficients (28.0±1.8%) than the pythons (21.1±0.3%) (Secor, 2009). This is largely because the catfish fillets are lower in energy (4.62 kJ g^{-1} wet mass) (Cox and Secor, 2010) compared with the rodent meals (7.0 kJ g^{-1} wet mass) (Ott and Secor, 2007), and hence a given SDA becomes a larger percentage of a catfish meal compared with a rodent meal of equal mass.

Gastric acid production

For the diamondback water snake and Burmese python, the production of HCl with feeding is evident from the documented profiles of their gastric pH following the ingestion of meals of the same type and relative size used in this study (Secor, 2003; Cox and Secor, 2010). During the 4–8 days of gastric digestion, water snakes and pythons maintained luminal pH at 2.0–3.0 and 1.5–2.0, respectively (Secor, 2003; Cox and Secor, 2010). The water snakes and pythons of this study exhibited similar postprandial rates of acidification of the Ussing chamber solution (quantified as the decrease in pH units per min) and acid production (μmol cm^{-2} h^{-1}). During our trials, the rate of acid production remained steady; a feature we believe is maintained throughout gastric digestion. Snakes not only need to produce enough HCl to drop luminal pH to 1.2–3.0 but they also have to continuously produce HCl to maintain pH at those levels. As the fish or rodent meal is being broken up, the exposed tissues and fluids acts as a continuous buffer to the HCl formed. For the python, postprandial profiles of gastric pH reveal that upon the completion of gastric digestion, HCl production ceases and luminal pH rapidly returns to neutrality. In this study, pythons had shut down HCl production by day 4 of digestion. At this time the stomach contained only a small mat of undigestible hair, the last remnant of the meal to be sent to the intestinal tract. This observation revealed the speed at which the python's stomach, recognizing this meal remnant, had shut down HCl production in anticipation of gastric emptying.

As observed in this study and previously, the python's stomach does not produce HCl between meals (Secor, 2003). For fasted pythons, gastric luminal pH is near neutrality, uniquely different from that of mammals, amphibians and fish; many of which maintain acid production, and thus a very acidic gastric environment, between meals (Youngberg et al., 1985; Savarino et al., 1988; Papastamatiou and Lowe, 2004) (S.S., unpublished data). The shutdown of acid production by pythons after completing digestion is proposed to be an energy-saving adaptation (Secor, 2003). Given the high cost of HCl production (one ATP expended per H^+ secreted), pythons are thereby reducing their energy expenditure during their extended periods of fasting (Reenstra and Forte, 1981).

It is presently unclear whether diamondback water snakes habitually maintain or reduce gastric acid production upon completing digestion. In a previous study, luminal gastric pH of four fasted water snakes measured immediately after they were killed varied from 3.3 to 7.0 (Cox and Secor, 2010). Three of these snakes possessed a non-acidic gastric lumen (pH>5.0). In the present study, the initial pH of the gastric tissue bath did not differ between fasted pythons and water snakes (Table 2). Whereas fasted python stomach tissues did not alter solution pH or produce HCl, the gastric samples from the three fasted water snakes acidified the solution, dropping its pH to 4.0 within an average of 69 min (tissues from fed snakes did so in an average of 50 min). From that point on, the fasted tissues continued to produce HCl at a rate equivalent to 62% of that exhibited by tissues from fed snakes (Table 2). While it is evident that water snakes do maintain gastric acid production while fasting, they are also able to reduce acid production in the absence of food.

Intestinal base secretion

For both water snakes and pythons, the acidic chyme has already begun to pass through the pyloric sphincter into the small intestine within 12 h of feeding (Cox and Secor, 2008; Cox and Secor, 2010). Upon entering the small intestine, the chyme is instantly neutralized. From the distal stomach to the first centimeters of the small intestine, chyme pH at day 1 of digestion increases from 2.68±0.13 to 6.79±0.13 for water snakes and from 3.44±0.17 to 6.71±.0.05 for pythons (Cox and Secor, 2010) (S.S., unpublished data). Neutralization of the acidic chyme originates from three sources: (1) bile released from the gall bladder, (2) HCO_3^- released from the pancreas, and (3) HCO_3^- released from the small intestine. Bile, although slightly acidic for Burmese pythons (pH=6.45±0.08; S.S., unpublished data), is continuously released into the intestinal lumen just distal to the pylorus during digestion. Bile and pancreatic HCO_3^-, likewise released just distal to the pylorus, are apparently the dominant buffers of the entering chyme. The contribution of intestinal HCO_3^- secretion to neutralizing the chyme appears relatively modest for water snakes and pythons. Although fed diamondback water snakes did experience an increase in intestinal base production, the increased rate of secretion was not enough to initiate acid titration. Digestion likewise generated intestinal base secretion for pythons, but only one intestinal segment from a fed individual released enough base to trigger acid titration and generate a measurable rate of HCO_3^- secretion (Table 2).

Compared with the snakes of this study, fish exhibit greater fasting and postprandial rates of intestinal HCO_3^- secretion. For digesting fish, intestinal HCO_3^- secretion potentially serves the dual role of buffering chyme and offsetting the alkaline tide (Taylor et al., 2007; Taylor and Grosell, 2009). The comparatively lower *in vitro* rates of intestinal HCO_3^- secretion for snakes may stem from the position from which the intestinal segments were sampled and/or the lack of a proper stimulus for secretion. Segments of small intestine were collected from the middle region of the proximal third of the small intestine. At this point, the contents had been neutralized enough that only a modest contribution of HCO_3^- from that site was required. Alternatively, luminal acid (lacking in our solution) may be necessary to stimulate HCO_3^- secretion as, observed for the European flounder, *Platichthys flesus* (Wilson and Grosell, 2003).

Gastric and intestinal electrophysiology

Fasting TEP and conductance values from the gastric and intestinal epithelia were in general similar between water snakes and pythons, and did not change significantly in the intestine in response to feeding. However, feeding resulted in a transient yet clear increase in gastric TEP for pythons, suggesting that the stimulation of acid secretion was paralleled by increased electrogenic transport. Although likely, it is unclear whether this increase in electrogenic transport is associated with acid secretion itself (basolateral anion exchange, apical K^+ leak and K^+/H^+ exchange) or due to the absorption and/or secretion of other electrolytes.

Gastric and intestinal oxygen consumption

For fasted and fed water snakes and pythons, rates of oxygen consumption by the mucosal epithelium tended to be greater than for the smooth muscle and connective tissue of the serosa (significantly so for fed pythons and for the small intestine of fasted pythons and fed water snakes). This was readily apparent for digesting pigs as $\dot{V}_{O_2}$ of their small intestinal mucosa was 20 times greater than that of their muscularis (Nyachoti et al., 2000). For water snakes, gastric mucosal and serosal $\dot{V}_{O_2}$ did not change with digestion, a feature due in part to both fasted and fed gastric tissues producing HCl in this study, and hence experienced similar metabolic costs. The python's postprandial doubling of gastric mucosal $\dot{V}_{O_2}$ and 35% increase in serosal $\dot{V}_{O_2}$ reflects their resumption of HCl production. Python routinely shut down HCl production upon the completion of digestion, maintain a fairly neutral gastric pH while fasting, and then commence acid production with the consumption of the next meal (Secor, 2003). For the active gastric mucosa, oxygen is consumed in the production of ATP, which fuels the proton pump's (H^+/K^+-ATPase) delivery of H^+ (against a strong concentration gradient) into the gastric crypts (Forte et al., 1980). The metabolic impact of HCl production was demonstrated by the administration of the proton pump inhibitor omeprazole to boa constrictors (*Boa constrictor*) prior to feeding; the normal postprandial metabolic response was delayed, presumably until new proton pumps were restored (Andrade et al., 2004).

Both species experienced an increase in $\dot{V}_{O_2}$ of the intestinal mucosa with feeding; a relatively modest 50% for water snakes and a more impressive 200% increase for the pythons. During digestion for both snakes, mucosal $\dot{V}_{O_2}$ was double that of serosal $\dot{V}_{O_2}$, suggestive of a greater activity by the epithelium. Contributors to the postprandial increase in mucosal $\dot{V}_{O_2}$ would include increased rates of protein synthesis, elevated activities of Na^+/K^+-ATPase, and increased Na^+-coupled nutrient transport (McBride and Kelly, 1990: Cant et al., 1996). Because pythons downregulate intestinal function with fasting and water snakes do not, the larger postprandial increase in intestinal $\dot{V}_{O_2}$ for pythons stems from the upregulation of their quiescent intestine (Secor and Diamond, 1995; Cox and Secor, 2008; Cox and Secor, 2010). The upregulation of intestinal function for pythons is largely due to the postprandial lengthening (by as much as 6-fold) of their intestinal microvilli (Starck and Beese, 2001; Lignot et al., 2005). With an enlarged surface area and many more enzymes and transporters to support, there is a compensatory increase in $\dot{V}_{O_2}$. The lack of any postprandial change in microvillus length for water snakes can explain the absence of any regulation of intestinal function with feeding and fasting and hence the more modest postprandial increase in oxygen consumption (Cox and Secor, 2010).

The python's downregulation of gastric and intestinal function with fasting is responsible for the significantly lower $\dot{V}_{O_2}$ ($ml\,g^{-1}\,h^{-1}$) of their stomachs and intestines compared with those of fasted water snakes. By integrating an organ's $\dot{V}_{O_2}$ ($ml\,g^{-1}\,h^{-1}$) with its mass and assuming constant $\dot{V}_{O_2}$ throughout the organ (stomach or small intestine), the cumulative $\dot{V}_{O_2}$ of the stomach and small intestine of fasted water snakes (combined mass=2.39±0.31% of body mass) was 0.94±0.17 $ml\,h^{-1}$, equivalent to 8.6% of their SMR. For fasted pythons, which averaged 50 g heavier than water snakes, combined $\dot{V}_{O_2}$ of the stomach and small intestine (2.84±0.18% of body mass) was 0.45±0.05 $ml\,h^{-1}$ (5.6% of SMR). Fasted pythons possessed both lower SMR and a lower combined metabolic rate of the stomach and small intestine (independent of SMR and organ mass) compared with fasted water snakes.

For pythons, feeding generated an increase in the metabolic rate of both the stomach and intestine and an increase in the mass of the small intestine. Combined stomach and small intestine masses at day 2 for water snakes and at day 3 for pythons equaled 3.95±0.23% and 3.26±0.10% of their body masses, respectively. At day 2 for water snakes, stomach and small intestine $\dot{V}_{O_2}$ had increased to 1.21±0.14 ml $O_2\,h^{-1}$ (a 29% increase compared with fasting), equivalent at that time to 3.1% of the snakes' metabolic rate (which had increased 4-fold). For pythons at day 3, combined $\dot{V}_{O_2}$ of the stomach and small intestine had risen to 1.85±0.17 ml $O_2\,h^{-1}$ (4.11-fold of fasting), equivalent to 2.6% of whole-animal metabolic rate (which has increased by 8.9-fold). In the transition from fasting to peak digestion, GI metabolism of both species increased, but not at the same pace as overall metabolic rate.

Intuitively, GI $\dot{V}_{O_2}$ would increase after feeding with a greater scope than whole-animal $\dot{V}_{O_2}$, given that much of the rise in $\dot{V}_{O_2}$ is presumably generated by the active gut. The metabolic response to digestion and assimilation, SDA, can be partitioned into preabsorptive and postabsorptive events (Secor, 2009). The general opinion is that SDA largely reflects the postabsorptive costs of nutrient conversion and protein synthesis (Secor, 2009). For animals that consume large intact meals and are continuously producing HCl for days (e.g. snakes, some anurans and deep sea fish), the preabsorptive costs of gastric performance would contribute comparably more to their SDA (Gartner et al., 1997; Secor, 2003; Secor, 2005b) (but see Wang et al., 2006). At day 1 for the python with only 10% of the meal absorbed, the 4.1-fold increase in GI metabolism nearly matched the 4.7-fold increase in whole-animal metabolism. In the days following, the relative increase in whole animal $\dot{V}_{O_2}$ outpaced the increase in GI $\dot{V}_{O_2}$. For the python, it has been suggested that early in digestion (<1 day) the increase in preabsorptive activity (reflected in the increase in gastric and intestinal metabolism) is responsible for the overall increase in whole-animal metabolic rate; however, as more of the meal is absorbed (>1 day), the postabsorptive costs of nutrient assimilation add to the preabsorptive costs, thereby increasing whole-animal $\dot{V}_{O_2}$. For water snakes at day 2, 65% of the ingested meal had been absorbed; thus, the 300% increase in snake $\dot{V}_{O_2}$ illustrates the dominant input of postabsorptive activities at that time.

It may be questioned whether we accurately measured gastric and intestinal metabolism. Hypothetically our tissues were damaged when extracted and placed in the Ussing chambers, and without their vascular supply they rapidly deteriorated during functional and metabolic measurements. Several lines of evidence have led us to believe that this was not the case. First, fasted rats (*Rattus novegicus*) possess intestinal mass-specific $\dot{V}_{O_2}$ (1.01 $ml\,g^{-1}\,h^{-1}$) that nearly matches basal metabolic rates (1.10 $ml\,g^{-1}\,h^{-1}$) (Field et al., 1939). In our study, mass-specific intestinal $\dot{V}_{O_2}$ of fasted water snakes and pythons was 3.8 and 2.6 times greater, respectively, than mass-specific SMR, indicating that these tissues were viable. Second, intestinal preparations from the gulf toadfish *O. beta* exhibited similar fasting and postprandial $\dot{V}_{O_2}$ values as those from pythons in this study and remained active and stable for at least 5 h under similar experimental conditions (Grosell and Genz, 2006; Taylor and Grosell, 2009). Third, gastric tissues from fasted and fed water snakes and from fed pythons were able to maintain a steady rate of acid production for several hours. Added to this, one python's intestinal tissue triggered acid titration after 70 min of incubation and continued secreting base for another 30 min before the experiment was terminated. And fourth, intestinal tissue from a fed python was left in the metabolic setup for an additional hour, during which it continued to deplete oxygen from the solution until none

was left (Fig. 5A). For this tissue, rates of oxygen consumption only began to decrease when the last of the oxygen in the solution was consumed (Fig. 5B). Still, our *in vitro* measurements of $\dot{V}_{O_2}$ may under-represent *in vivo* rates given the absence of nutrient, hormonal and/or neural stimulation of the tissues within the Ussing chambers (Watford et al., 1979).

An integrated perspective

Upon the completion of digestion, pythons severely downregulate gastric acid production, pancreatic enzyme secretion, intestinal nutrient uptake, hydrolase activity and base secretion (Secor and Diamond, 1995; Cox and Secor, 2008). Feeding triggers, by direct contact with meal nutrients and/or by neurohumoral stimulation, the rapid upregulation of gastric and intestinal function and hypertrophy of the intestinal mucosa (Secor and Diamond, 1995; Secor et al., 2000; Secor et al., 2001; Secor et al., 2002; Lignot et al., 2005). If the wide regulation of GI performance is an adaptive trait to complement a feeding ecology characterized by predicted long episodes of fasting, then there should predictably be a selective incentive. That incentive is a depression of metabolic rate that allows the animal to survive long episodes of fasting on stored nutrients (e.g. fat bodies and glycogen). We demonstrated for the python that one mechanism underlying a depressed SMR is a significant lowering of organ function and hence metabolism. It is expected, though yet to be determined, that other organs (e.g. liver, pancreas and kidneys) likewise downregulate form and function with fasting, and that their reduced metabolism also contributes to a depressed SMR. This adaptive repertoire apparently does not exist for the frequently feeding diamondback water snake. Rather, they maintain function of the intestinal tract and other organs (suggested by the lack of any significant change in mass) during fasting and hence between meals experience both higher tissue metabolic rates and higher SMR. For these snakes, the maintenance of intestinal function, and thus elimination of regulatory processes, selectively outweighs the energetic benefits of downregulation.

While it may be premature to infer among snakes an adaptive interplay of tissue function, metabolism and feeding habits from this two-species study (Garland and Adolph, 1994), the study does encourage attention to the following questions. (1) What has been the target(s) of selection in the adaptive regulation of GI performance and metabolism for snakes? Candidates include at least the cellular and molecular mechanisms underlying gastric acid production and intestinal base secretion, cellular growth, microvillus growth, nutrient transport and hydrolase activities. (2) How conserved with respect to phylogeny and/or feeding habits is the adaptive cascade of downregulation of GI function that results in a depression in tissue metabolism which accumulates in a reduce basal metabolism that enhances survival during prolonged fasts? We know that infrequently feeding snakes possess relatively low SMRs and that they downregulate intestinal performance following processing of a meal (Secor, 2005a; Ott and Secor, 2007). This study has shown for one infrequently feeding snake that there is a corresponding decrease in GI metabolism. (3) Similar questions could apply to frequently feeding snakes and the linear dependence of intestinal maintenance on intestinal metabolism and SMR. Clearly the wide or modest regulation of GI performance for snakes is founded in a complexly integrated selected process.

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