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Metabolic and digestive response to food ingestion in a binge-feeding lizard, the Gila monster (*Heloderma suspectum*)

C. M. Christel[1,*], D. F. DeNardo[1] and S. M. Secor[2]

[1]*Arizona State University, School of Life Sciences, PO Box 874501, Tempe, AZ 85287, USA and* [2]*University of Alabama, Department of Biology, 420 Biology Building, Tuscaloosa, AL 35487, USA*

*Author for correspondence (e-mail: churst@cc.edu)

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Summary

The gastrointestinal tract possesses the capacity to change in form and function in response to fasting and feeding. Such plasticity can be dramatic for species that naturally experience long episodes of fasting between large meals (e.g. sit-and-wait foraging snakes, estivating anurans). By contrast, for active foraging species that feed more frequently on smaller meals, gastrointestinal responses are more modest in magnitude. The Gila monster *Heloderma suspectum* is an active foraging lizard that feeds infrequently on meals weighing up to one-third of its body mass. Additionally, Gila monsters possess a species-specific salivary peptide, exendin-4, which may be involved in the regulation of metabolic and digestive performance. To investigate the adaptive postprandial response of Gila monsters and the potential regulatory role of exendin-4, we measured metabolic and intestinal responses to feeding in the presence or absence of circulating exendin-4. Following the consumption of rodent or egg meals equivalent to 10% of lizard body mass, metabolic rates peaked at 4.0- to 4.9-fold of standard metabolic rates and remained elevated for 5–6 days. Specific dynamic action of these meals (43–60 kJ) was 13–18% of total meal energy. Feeding triggered significant increases in mucosal mass, enterocyte width and volume, and the upregulation of D-glucose uptake rates and aminopeptidase-N activity. Total intestinal uptake capacity for L-leucine, L-proline and D-glucose were significantly elevated within 1–3 days after feeding. Whereas the absence of circulating exendin-4 had no impact on postprandial metabolism or the postprandial response of intestinal structure and nutrient uptake, it significantly increased intestinal aminopeptidase-N activity. Within the continuum of physiological responses to feeding and fasting, Gila monsters occupy an intermediate position in experiencing moderate, though significant, regulation of intestinal performance with feeding.

Key words: aminopeptidase-N, digestion, exendin-4, intestinal nutrient transport, reptile, specific dynamic action.

Introduction

Tissue form and function vary in response to temporal changes in physiological demands. Such plasticity is well exemplified by the gastrointestinal (GI) tract, especially for species that experience large fluctuations in digestive demand (Piersma and Lindström, 1997). Recognized examples include animals that hibernate, estivate or, as a function of their sit-and-wait foraging tactics, experience long episodes of fasting between meals (Carey, 1990; Secor and Diamond, 2000; Secor, 2005a). In each of these cases the GI tract is quiescent during months of fasting and, following feeding, rapidly restores the ability to digest and assimilate a meal. For several vertebrate taxa, the capacity to regulate GI performance is linked with feeding habits. For sit-and-wait foraging snakes and anurans that estivate, intestinal performance is severely downregulated when fasting and then quickly upregulated following feeding (Secor and Diamond, 2000; Secor, 2005a; Ott and Secor, 2007a). In contrast, active foraging snakes that feed relatively frequently and anurans that do not estivate experience little downregulation of intestinal function with fasting, and hence only modestly upregulate intestinal performance with feeding (Secor and Diamond, 2000; Secor, 2005a; Secor, 2005b). The broad regulation of GI performance for animals that naturally experience long periods of fasting is hypothesized to be an adaptive energy-conserving trait (Secor, 2005b). During periods of fasting, the GI tract is maintained at a basal level to conserve energy as it does not need to be functional during this time. When food is ingested, the GI tract upregulates in morphology and function to digest and absorb the meal. The energy needed for this upregulation is less than that needed to maintain the GI tract in a functional state over the fasting period, resulting in net energy conservation. Likewise, the modest regulation of GI performance for frequently feeding animals is proposed to be optimal for energy efficiency (Secor, 2005b).

Large magnitudes of postprandial response in GI structure and function have been documented for sit-and-wait foraging pythons. With feeding, these snakes experience dramatic increases in gastric acid production, a five- to tenfold increase in intestinal nutrient transport, a fourfold increase in intestinal aminopeptidase-N activity, a doubling of small intestinal mass, and a fivefold increase in intestinal microvillus length (Secor and Diamond, 1995; Starck and Beese, 2001; Secor, 2003:

Lignot et al., 2005; Ott and Secor, 2007a). It is predicted that this suite of postfeeding responses is orchestrated in part by several regulatory peptides. Evidence of hormonal interaction for the Burmese python *Python molurus* is the rapid postprandial increase in plasma concentrations of several GI peptides (CCK, GIP, glucagon) and the concurrent decline of those peptides in their source tissues (Secor et al., 2001).

While the broad regulation of digestive performance has been observed for infrequently feeding snakes, it remains to be seen whether this apparent adaptive response is characteristic of other infrequently feeding reptiles. One well-suited candidate to explore the generality of this response is the Gila monster *Heloderma suspectum*. The Gila monster, one of two venomous lizard species in the world, can fast for months in nature and then binge on meals (lizard and bird eggs or neonate rodents and rabbits) that may exceed a third of the lizard's body mass (Beck, 2005). Thus, like the previously studied pythons, Gila monsters would be predicted to significantly regulate intestinal performance with feeding and fasting. This regulatory response would likewise be triggered by circulating peptides. Interestingly, the Gila monster possesses a unique peptide in its saliva, exendin-4, which when administered to diabetic mammals stimulates insulin release, resulting in a decrease in plasma glucose concentrations (Young et al., 1999; Szayna et al., 2000). Although the plasma concentration of exendin-4 increases after feeding for the Gila monster, it does not appear to regulate postprandial concentration of plasma glucose or triglycerides for this lizard (Christel and DeNardo, 2006; Christel and DeNardo, 2007).

Given that Gila monsters typically feed infrequently in the wild and that they possess a unique peptide that is released with feeding, we set out to explore the extent that they regulate intestinal performance with feeding and fasting and the potential role of exendin-4 in regulating that response. We designed this study to characterize and compare among lizards with and without elevated exendin-4 levels: (1) the postprandial metabolic response and cost of meal digestion, (2) the change in intestinal nutrient uptake and hydrolase activity with feeding, (3) the postprandial change in intestinal morphology and organ mass, and (4) the magnitude that intestinal performance is regulated.

Materials and methods

Animals and housing

We used 18 adult Gila monsters *Heloderma suspectum* Cope 1869 (11 males, mean mass 468 g, range 401–632 g, and 7 females, mean mass 490 g, range 332–605 g), acquired from the Arizona Game and Fish Department captive collection of non-releasable animals. Animals were transported by air to the University of Alabama and maintained prior to experimentation in large fiberglass cages (67 cm×30 cm×18 cm), 4–5 Gila monsters per cage, at a temperature of 25–28°C. Within the cages, Gila monsters were provided with refugia and water. Prior to study, Gila monsters were fasted for a minimum of 30 days to ensure that they were postabsorptive.

Experimental procedures

Gila monsters experience significant elevations in plasma exendin-4 concentrations after biting or feeding on rodent prey; however, if force-fed rodents while under anesthesia, they do not increase plasma exendin-4 concentrations (Christel and DeNardo, 2006). Likewise, when feeding upon chicken egg white and yolk they do not experience an elevation in plasma exendin-4 (Christel and DeNardo, 2006). To examine the metabolic responses of Gila monster to different meals and to circulating plasma exendin-4 concentrations, we used the following three meal treatments: (1) pre-killed neonate rats (~20 g) fed voluntarily, (2) pre-killed neonate rats fed under anesthesia, and (3) chicken egg white and yolk fed voluntarily. For the second meal treatment, which was designed to prevent endogenous exendin-4 release, we lightly anesthetized each Gila monster by placing it in a chamber containing isoflurane until it was unresponsive to touch. We then used a bird speculum to keep the mouth open and a pair of hemostats to push the pre-killed neonate rat into the Gila monster's stomach. For each meal treatment we used six Gila monsters and meal mass was equal to 10.02±0.01% of Gila monster body mass.

To explore the postprandial responses of Gila monsters for intestinal function and morphology, and the potential regulatory role of exendin-4 for those responses, we compared intestinal structure, nutrient uptake and hydrolase activity among four feeding treatments. Twelve Gila monsters were divided equally among four treatment groups so that there would be no significant difference in mean body mass among treatments. The four treatment groups were: (1) after a 30-day fast with no expected circulating exendin-4 (Fasted); (2) 1 day following the voluntary consumption of a pre-killed neonate rat meal with expected elevated plasma exendin-4 levels (1DPF); (3) 1 day following the force-feeding under anesthesia (described above) of a pre-killed neonate rat with no expected release of exendin-4 (1DPF-anes); and (4) 3 days following the voluntary consumption of pre-killed neonate rat meal with expected elevated plasma exendin-4 levels (3DPF). For the three feeding treatments, meal mass was equivalent to 10.01±0.004% of Gila monster body mass. After treatment, Gila monsters were killed by severing their spinal cord, immediately posterior to the head. A mid-ventral incision was made to expose the internal organs, which were removed and weighed. For fed animals, the stomach and small intestine were emptied of their contents and reweighed. The difference between organ full mass and empty mass was recorded as the wet mass of organ contents.

Metabolic rate and specific dynamic action (SDA)

We quantified pre- and postprandial metabolism of Gila monsters by measuring rates of oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) using closed-system respirometry as described by Secor (Secor, 2003). Gila monsters were placed individually into opaque respirometry chambers (volume 9 l) and maintained at 30°C within an environmental chamber. Each respirometry chamber was fitted with incurrent and excurrent air ports, each attached to a three-way stopcock. With the exception of sampling periods, air was continuously pumped into chambers through the incurrent air port.

For each measurement of gas exchange, we withdrew a 50 ml air sample from the excurrent air port, and closed both ports to seal the chamber. 0.5–1 h later, the excurrent air port was opened and a second 50 ml air sample was withdrawn. Air

samples were pumped (125 ml min^{-1}) through a column of water absorbent material (Drierite™; W. A. Hammond Drierite Co., Xenia, OH, USA) and CO_2 absorbent material (Ascarite II; Thomas Scientific, Swedesboro, NJ, USA) into an O_2 analyzer (S-3A/II; AEI Technologies, Pittsburgh, PA, USA) and through a column of water absorbent material into a CO_2 analyzer (CD-3A; AEI Technologies). We calculated whole-animal (ml h^{-1}) and mass-specific (ml g^{-1} h^{-1}) rates of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$, corrected for standard pressure and temperature as described previously (Vleck, 1987).

We began the metabolic trial by measuring rates of gas exchanges of each Gila monster twice a day (at ~08:00 h and 20:00 h) for 4 days. We assigned for each Gila monster its standard metabolic rate (SMR) as the lowest $\dot{V}_{O_2}$ and accompanied $\dot{V}_{CO_2}$ measured over those days. Following SMR determination, each Gila monster was fed, returned to its respirometry chamber, and measurements resumed at 12 h intervals (~08:00 h and 20:00 h) for 3 days and thereafter at 1-day intervals (~08:00 h) for 7 more days.

We characterized the postprandial metabolic response to meal digestion, absorption and assimilation for each animal by quantifying the following seven 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) respiratory exchange ratio (RER) calculated as $\dot{V}_{CO_2}/\dot{V}_{O_2}$; (5) duration, the time after feeding that $\dot{V}_{O_2}$ was significantly elevated above SMR; (6) SDA (specific dynamic action), the total energy expenditure above SMR over the duration of significantly elevated $\dot{V}_{O_2}$; and (7) 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 O_2 consumed, assuming that the dry matter of the catabolized 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 the rodent and egg meals was calculated by multiplying meal mass by its specific energy equivalent (kJ g^{-1} wet mass) determined by bomb calorimetry. Five neonate rat and five eggs (minus the shell) were weighed (wet mass), dried, reweighed (dry mass), ground to a fine powder, and pressed into pellets. Three pellets from each individual rat or egg were ignited in a bomb calorimeter (1266, Parr Instruments Co., Moline, IL, USA) to determine energy content (kJ g^{-1}). For each meal, we determined wet-mass energy equivalent as the product of dry mass energy content and dry mass percentage. The neonate rats had a dry mass percentage of 26.0±0.3% and an energy equivalent of 6.82±0.13 kJ g^{-1} wet mass, whereas the shell-less eggs had a dry mass percentage of 24.6±0.3% and an energy equivalent of 7.14±0.17 kJ g^{-1} wet mass.

Intestinal morphology and organ masses

We examined the effects of feeding treatment on small intestinal morphology by measuring intestinal mass, intestinal length, mucosa and muscularis/serosa thickness, and enterocyte dimensions from fasted and fed Gila monsters. For each Gila monster, we weighed the emptied small intestine and measured its length. A 1 cm segment from the middle region was fixed in 10% neutral-buffered formalin solution, embedded in paraffin, and cross-sectioned (6 μm slices). Several cross-sections were placed on a glass slide and stained with Hematoxylin and Eosin. The thickness of the mucosa and muscularis/serosa layers and the height and width of ten enterocytes were measured at ten sites on each cross-section using a light microscope and video camera linked to a computer and image-analysis software (Motic Image Plus, British Columbia, Canada). For each Gila monster we report the average thickness of the mucosa and muscularis/serosa layers, the average height and width of enterocytes, and the average enterocyte volume, calculated using the formula for a cube (enterocyte $width^2 \times height$). To determine treatment effects on the mass of other organs, we determined the wet mass of the heart, lungs, liver, empty stomach, pancreas, empty large intestine and kidneys immediately upon their removal, dried each organ at 60°C for 2 weeks, and reweighed each to obtain dry mass.

Intestinal aminopeptidase-N activity

For fasted and fed Gila monsters, we measured the activity of the brush border bound hydrolase, aminopeptidase-N (APN; EC 3.4.11.2) from the proximal third of the small intestine, following the procedure of Wojnarowska and Gray (Wojnarowska and Gray, 1975) and used previously on pythons (Ott and Secor, 2007a). 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). Scraped mucosa from intestinal segments was homogenized in PBS (1:250 dilutions) on ice. We used leucyl-β-naphthylamide (LNA) as the substrate and *p*-hydroxymercuribenzoic acid to inhibit nonspecific cytosol peptidases to quantify APN activity. Absorbance of the product resulting from the hydrolysis of LNA was measured spectrophometrically (DU 530, Beckman Coulter, Fullerton, CA, USA) at 560 nm and compared to a standard curve developed with β-naphthylamine. We quantified APN activity as μmol substrate hydrolyzed min^{-1} g^{-1} mucosal protein. Protein concentration of the homogenate was determined using Bio-Rad (Hercules, CA, USA) Protein Assay kit based on the Bradford method (Bradford, 1976).

Intestinal nutrient uptake

We measured nutrient transport rates across the intestinal brush border membrane of fasted and fed Gila monsters using the everted sleeve technique as described by Karasov and Diamond (Karasov and Diamond, 1983) and Secor and Diamond (Secor and Diamond, 2000). This method can be performed on the intestines of lizards and snakes without damaging the intestinal mucosal (Ott and Secor, 2007a; Tracy and Diamond, 2005). The small intestine was removed, cleared of its contents, everted and divided into equal-length thirds (proximal, middle and distal). Each third was weighed and then 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) (Secor et al., 1994). From intestinal segments we

measured the total uptake (passive and carrier-mediated) of the amino acids L-leucine and L-proline, as well as the active, carrier-mediated uptake of D-glucose. Nutrient uptake rates were quantified as nanomol nutrient transported min^{-1} incubation mg^{-1} segment wet mass. In addition, we quantified the intestine's total uptake capacity (reported as μmol min^{-1}) for each nutrient by summing together the product of segment mass (mg) and mass-specific rates of nutrient uptake (nmol min^{-1} mg^{-1}) for the proximal, middle and distal segments.

Statistical analysis

For each of the three SDA trials, we used repeated-measures analysis of variance (RM-ANOVA) to test for significant effects of time (before and after feeding) on $\dot{V}_{O_2}$. Concurrently, we used *post hoc* pairwise mean comparisons (Tukey test) to determine when postfeeding $\dot{V}_{O_2}$ was no longer significantly different from SMR, and to identify significant differences in $\dot{V}_{O_2}$ between sampling times. To test for meal treatment 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. To identify positional effects on nutrient uptake rates, we employed RM-ANOVA for each treatment (fasted and fed). Among treatments we used ANOVA to compare nutrient uptake rates for each intestinal position and APN activity for the middle segment, and ANCOVA to compare intestinal nutrient uptake capacities. We followed significant ANOVA and ANCOVA results with *post hoc* tests to identify significant differences between meal treatments. We calculated each statistical analysis using SAS and designated the level of statistical significance as P=0.05. Mean values are reported as mean ± 1 s.e.m.

Results

Metabolic rate and SDA

Among meal treatments there was no significant (all P>0.82) difference in either body mass or SMR (Table 1). Feeding for each treatment generated significant (all P<0.0001) increase in gas exchange, as $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ rose sharply to peak at 36 h postfeeding and then declined more gradually thereafter (Fig. 1). Although there was no significant difference in postprandial peak $\dot{V}_{O_2}$ among meal treatments, the factorial scope of peak $\dot{V}_{O_2}$ did vary significantly (P=0.016) among treatments as the scope from the egg meals (4.03) was significantly less than the scopes for either rat meals (4.80 and 4.90, Table 1). For the egg meal, RER varied significantly (P=0.003) among sampling periods, ranging from 0.68 to 0.81; however, there was no significant variation in RER among meals when calculated at the postprandial peak $\dot{V}_{O_2}$ (Table 1). Gila monsters digesting the egg meals experienced significantly elevated metabolic rates for 5 days, whereas lizards digesting the rat meals maintained elevated $\dot{V}_{O_2}$ for 6 days. SDA, the overall cost of digestion and assimilation, varied (P=0.048) among meals, as the cost of digesting either rodent meals was significantly greater than that of the egg meal (Table 1). Likewise, the SDA coefficient (SDA as a percentage

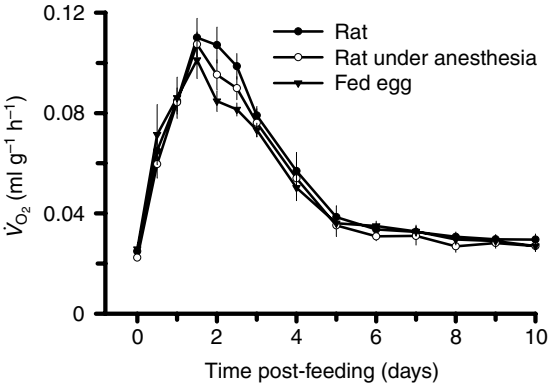

Fig. 1. Mean rates of oxygen consumption ($\dot{V}_{O_2}$) of *Heloderma suspectum* prior to (day 0) and following the consumption of rodent meals, rodent meals fed under anesthesia, or egg meals equaling 10% of lizard body mass (N=6 for each meal). Values are means ±1 s.e.m.

Table 1. *Body mass, meal size, standard metabolic rate (SMR) and post-feeding metabolic measures of peak oxygen consumption, scope of peak, duration, specific dynamic action (SDA) and SDA coefficient of Gila monsters in response to three feeding treatments*

Variable	Fed egg	Fed rat	Fed rat under anesthesia	F	P
Body mass (g)	460±46	480±20	491±34	0.20	0.822
Meal size (g)	46.2±4.6	48.0±2.0	49.1±3.4	0.18	0.840
SMR (ml O_2 h^{-1})	11.8±1.4	12.0±1.1	11.0±0.9	1.08	0.366
SMR (ml O_2 g^{-1} h^{-1})	0.026±0.002	0.025±0.002	0.022±0.001	1.14	0.347
SMR (ml CO_2 h^{-1})	9.44±1.04	9.12±0.77	8.77±0.76	1.79	0.202
SMR (ml CO_2 g^{-1} h^{-1})	0.021±0.001	0.019±0.001	0.018±0.001	1.98	0.172
Peak $\dot{V}_{O_2}$ (ml h^{-1})	46.5±4.2	56.4±3.9	54.2±4.9	1.84	0.195
Peak $\dot{V}_{O_2}$ (ml g^{-1} h^{-1})	0.103±0.007	0.117±0.006	0.110±0.005	1.63	0.229
Scope (peak $\dot{V}_{O_2}$/SMR)	4.03±0.24[a]	4.80±0.24[b]	4.90±0.09[b]	5.49	0.016
RER ($\dot{V}_{CO_2}$/$\dot{V}_{O_2}$)	0.707±0.019	0.726±0.027	0.762±0.031	1.13	0.349
Duration (days)	5	6	6		
SDA (kJ)	43.2±4.4[a]	59.9±5.2[b]	55.4±8.0[a,b]	3.78	0.048
SDA (kJ kg^{-1})	94.0±2.8[a]	124±7[b]	111±9[a,b]	4.97	0.022
SDA coefficient (%)	13.1±0.4[a]	18.2±1.1[b]	16.2±1.3[a,b]	6.72	0.008

$\dot{V}_{O_2}$, rate of oxygen consumption.
Values are means ± s.e.m. (N=6 animals/treatment). Different letters represent significantly different means (see text for details).

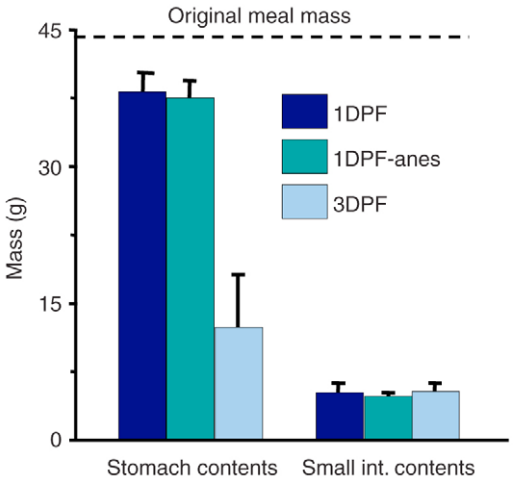

Fig. 2. Mass of stomach and small intestinal contents of *Heloderma suspectum* at 1 and 3 days following the consumption of rodent meals equal in mass to 10% of lizard body mass. For each meal treatment, ingested meals averaged 44.2 g. Meal treatments include 1 day postfeeding of rodent meal (1DPF), 1 day postfeeding of rodent meal under anesthesia (1DPF-anes), and 3 days postfeeding of rodent meal (3DPF). Values are means ±1 s.e.m.

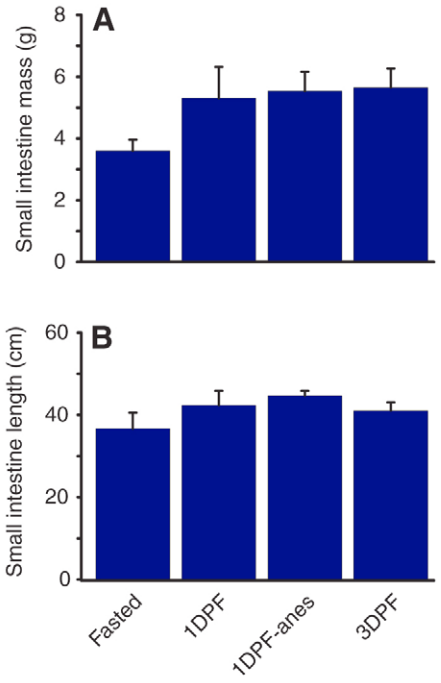

Fig. 3. Small intestine mass (A) and length (B) of fasted and fed *Heloderma suspectum*. For details of meal treatments, see Fig. 2. Values are means ±1 s.e.m., *N*=3 for each fasted and fed treatment.

of meal energy) varied significantly (P=0.008) as the relative cost of egg meal digestion was less than that of rat meal digestion (Table 1).

Rate of digestion

By 1 day postfeeding (combining 1DPF and 1DPF-anes treatments), the stomach contained 86.2±2.9% of the ingested meal, while the small intestine held 11.3±0.8% of the ingested meal (Fig. 2). At day 3 of digestion, the mass of stomach contents had further decreased to 28.3±14.5% of the consumed meal mass, and small intestinal contents equaled 12.0±1.9% of meal mass (Fig. 2).

Tissue mass and morphology

Small intestinal wet mass and length did not differ significantly (all P>0.21) among the fasted and fed treatments (Fig. 3). It is worth noting that the small intestine of fed animals averaged 52.5% heavier and 16.3% longer than the intestines of fasted lizards. We observed no significant variation among treatments in the thickness of the muscularis/serosa layer of the small intestine (Fig. 4). In contrast, the mucosal layer was significantly (all P<0.019) thicker for each of the fed treatments compared to fasted lizards (Fig. 4). Although the height of intestinal enterocytes did not vary significantly among fasted and fed treatments, feeding did have a significant effect on enterocyte width and volume (all P<0.02) (Fig. 5). Within 24 h after feeding, enterocyte width and volume had increased by 74% and 61%, respectively. By day 3 of digestion, lizards experienced a further 82% and 57% increase in cell width and volume, respectively (Fig. 5). We did not detect any significant postfeeding increase in the wet or dry mass of other organs, although on average the pancreas and kidneys were 49.3% and 47.8% heavier, respectively, in fed lizards compared to fasted lizards (not shown).

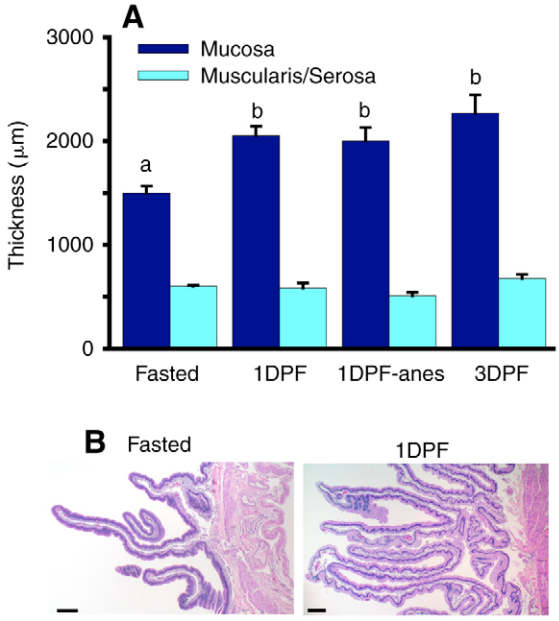

Fig. 4. (A) Thickness of the proximal intestine mucosa and muscularis/serosa layers and (B) micrographs of the intestinal epithelium of fasted and fed *Heloderma suspectum* (1DPF). For details of meal treatments, see Fig. 2. Scale bars, 200 μm; Values in A are means ±1 s.e.m., *N*=3 for each fasted and fed treatment. Different letters above the bars denote significant differences between means (for P values, see text). For each fed treatment intestinal mucosa had significantly increased in thickness, whereas the muscularis/serosa layer did not change.

Intestinal aminopeptidase-N activity

Intestinal APN activity varied significantly (P=0.001) among fasted and fed treatments, as lizards for each of the fed treatments expressed significantly (all P<0.01) greater APN activity compared to fasted lizards (Fig. 6). We also observed significantly (P<0.04) greater APN activity for lizards of the 1DPF-anes treatment compared to either the 1DPF or 3DPF treatment.

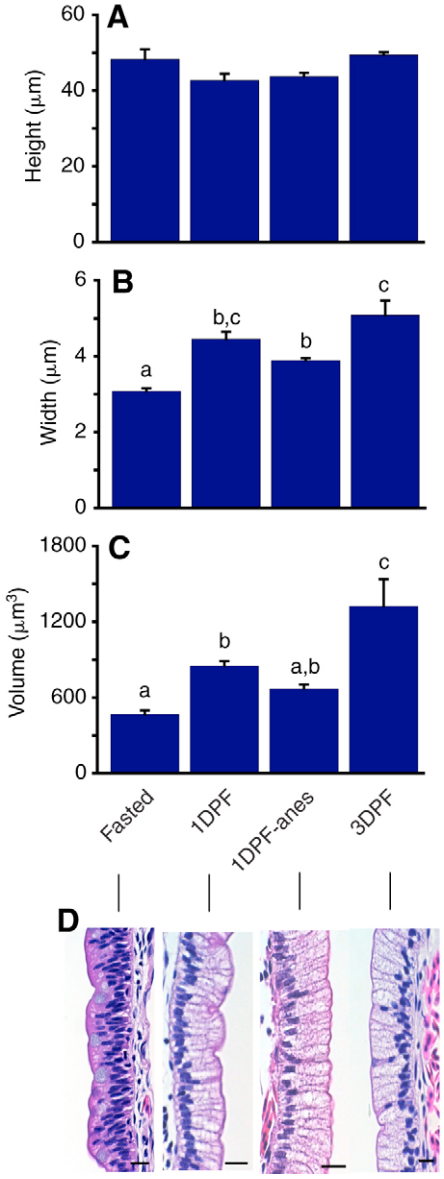

Fig. 5. Height (A), width (B), volume (C) and micrographs (D) of intestinal enterocytes from the proximal small intestine of *Heloderma suspectum*. Values are means ±1 s.e.m., N=3 for each fasted and fed treatment. Different letters above the bars denote significant differences between means. Scale bars in D, 20 μm. *Heloderma suspectum* experience a significant increase in enterocyte width and volume 1 day after feeding (1DPF), and a further increase in width and volume by day 3 of digestion (3DPF). For details of meal treatments, see Fig. 2.

Intestinal nutrient uptake

For 6 of the 12 assessments (3 nutrients × 4 feeding treatments), uptake rates varied significantly (all P<0.04) among the three intestinal positions. In each of these cases, uptake rates of the proximal segment were significantly (all P<0.04) greater than uptake rates of the distal segment. Regardless of intestinal position, uptake rates of L-leucine and L-proline did not significantly differ (all P>0.25) among fasted and fed treatments (Fig. 7). In contrast, uptake rates of D-glucose did vary significantly (all P=0.04) among treatments for the proximal and middle intestinal regions (Fig. 6). Proximal D-glucose uptake had significantly increased by twofold within 24 h after feeding, whereas the uptake of D-glucose by the middle region had a significant fivefold increase by day 3 of digestion.

Nutrient uptake capacity

While feeding-induced changes in segment masses and mass-specific uptakes were not statistically significant, the cumulative effect of these changes led to a significant increase in the total intestinal uptake capacity. Gila monsters experienced significant increases in total intestinal uptake capacities for each of the three studied nutrients. Intestinal L-leucine uptake capacities of 3DPF lizards were twice those calculated for fasted lizards (Fig. 8). For each of the fed treatments, L-proline uptake capacity had increased by 1.4–1.9% over the calculated capacity for fasted lizards (Fig. 8). Similarly, feeding generated significant increases in D-glucose uptake capacity (with the exception of the 1DPF-anes treatment), with three- and fourfold increases experienced 1 and 3 days postfeeding, respectively (Fig. 8).

Discussion

Gila monsters responded to feeding with an increase in metabolic rate and a modest upregulation of intestinal performance. Exendin-4 had no effect on postprandial metabolism or intestinal form but may affect APN activity. Below, we discuss and compare the Gila monsters' SDA response, gastric performance, postprandial changes in intestinal morphology and function, the role of exendin-4, and the adaptive significance of their intestinal response.

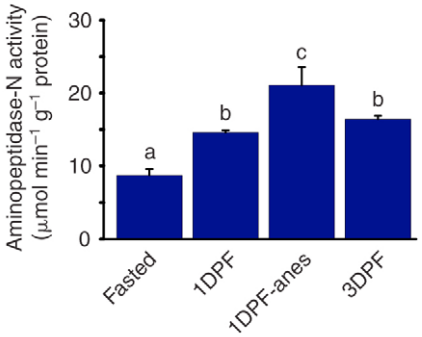

Fig. 6. Aminopeptidase-N activity of the proximal small intestine of fasted and fed *Heloderma suspectum*. Feeding resulted in a significant increase in aminopeptidase-N activity, which was further elevated for lizards that had consumed meals while under anesthesia. For details of meal treatments, see Fig. 2.

Metabolic response to prey ingestion

The postprandial metabolic profile of Gila monsters is typical of that of other lizards and other reptiles. As for most reptiles studied, postprandial rates of gas exchange increase rapidly to peak at 1–2 days postfeeding, before declining more slowly to prefeeding levels (Coulson and Hernandez, 1983; Secor and Phillips, 1997; Secor and Diamond, 1999; Secor and Diamond, 2000). The height and duration of the postprandial metabolic profile are impacted by meal size and type and the size and body temperature of the animal (Secor and Diamond, 1998; Toledo et al., 2003; Zaidan and Beaupré, 2003; Pan et al., 2005). Meals in this study weighed 10% of lizard body mass and generated 4.0- to 4.9-fold increases in metabolic rate for Gila monsters. For the lizards *Eumeces chinensis*, *Varanus albigularis*, *Tupinambis merianae* and *Angolosaurus skoogi*, meals of similar relative size (8.7, 9.9, 10.9 and 11% of body mass, respectively) resulted in 2.0-, 9.9-, 2.8- and 1.8-fold increases in metabolic rate (Clarke and Nicolson, 1994; Klein et al., 2006; Pan et al., 2005; Secor and Phillips, 1997). The large variation in metabolic scope among these lizards reflects, in part, the different type of meals that were consumed (frog flesh, rats, minced beef and plants, respectively).

In the present study, the digestion of the egg meal generated a smaller scope in peak $\dot{V}_{O_2}$ and a smaller SDA (by 24%) compared to the rodent meal. It is reasonable to assume that the egg white and yolk meals took less digestive effort than the intact rodent meals. Similarly for the Burmese python, the digestion and assimilation of homogenized rodents required 25% less energy than that of digesting intact rodents (Secor, 2003). The SDA coefficient, SDA quantified as a percentage of meal energy, likewise was significantly less for the egg meal,

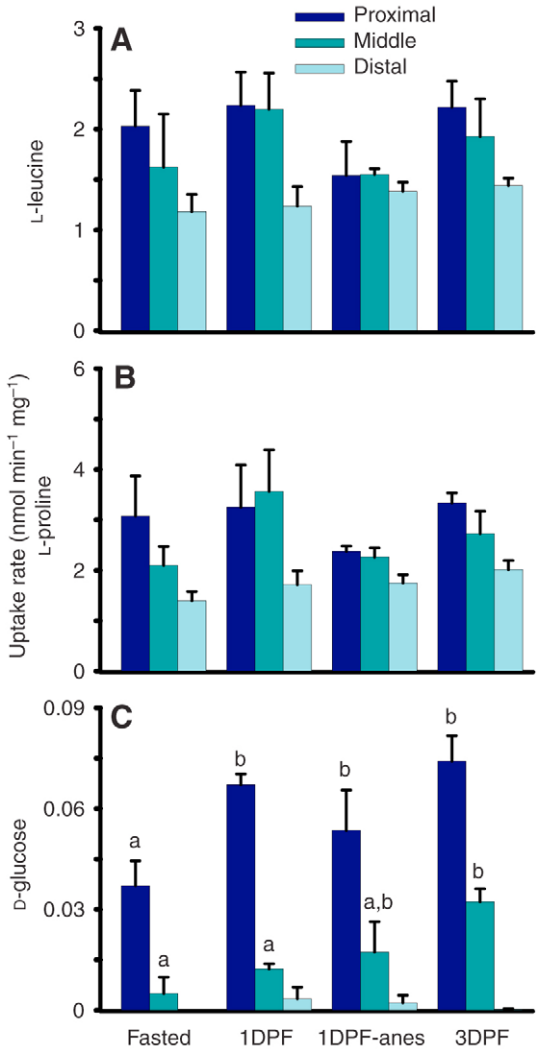

Fig. 7. Uptake rate of L-leucine (A), L-proline (B) and D-glucose (C) of the proximal, middle and distal portions of the small intestine of fasted and fed *Heloderma suspectum*. For details of meal treatments, see Fig. 2. Values are means ±1 s.e.m., *N*=3 for each fasted and fed treatment. Different letters above the bars denote significant differences between means (see text for *P* values). There was no detectable uptake of D-glucose for the distal small intestine of fasted lizards; therefore, no bar is shown. Lizards experienced no significant postprandial increase in L-leucine and L-proline uptake, whereas D-glucose uptake increased significantly with feeding in the proximal and middle small intestine.

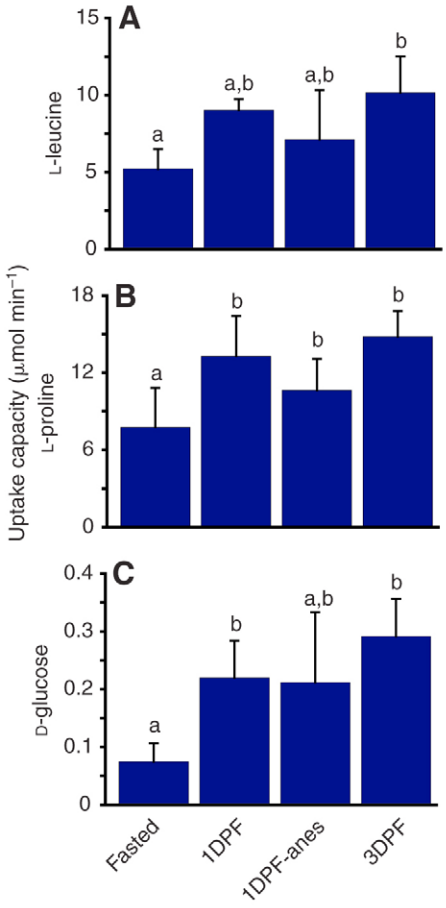

Fig. 8. Total intestinal uptake capacity of L-leucine (A), L-proline (B) and D-glucose (C) of fasted and fed *Heloderma suspectum*. For details of meal treatments, see Fig. 2. Values are means ±1 s.e.m., *N*=3 for each fasted and fed treatment. Different letters above the bars denote significant differences between means. Each feeding treatment experienced significant increases in uptake capacity for the three nutrients.

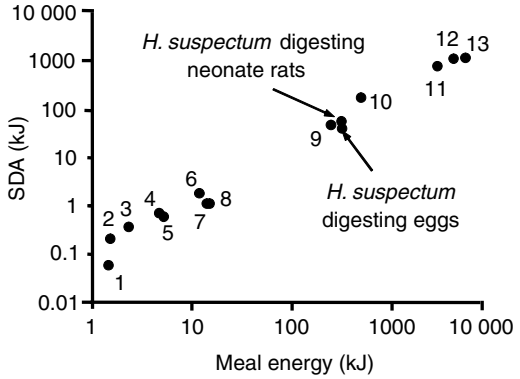

Fig. 9. Specific dynamic action (SDA, in kJ) plotted against meal energy (kJ) for 11 species of lizards. The log plot was used for convenience in visualizing the range of data. On average, SDA was equivalent to 17.5% of meal energy. Numbers signify the following species and their meal type: 1, *Sceloporus merriami* – cricket meal (Beaupré et al., 1992); 2, *Anolis carolinensis* – beef meal (Coulson and Hernandez, 1983); 3, *Sceloporus occidentalis* – cricket meal (Roe et al., 2005); 4, *Eulamprus tympanum* – mealworm meal (Robert and Thompson, 2000); 5, *Sphenomorphus indicus* – mealworm meal (Hong-Liang et al., 2004); 6, *Eumeces chinensis* – frog meal (Pan et al., 2005); 7, *Eumeces chinensis* – mealworm meal (Pan et al., 2005) ;8, *Eulamprus quoyii* – mealworm meal (Iglesias et al., 2003); 9, *Varanus exanthematicus* – rodent meal (Hicks et al., 2000); 10, *Tubinambis merianae* – beef meal (Klein et al., 2006); 11, *Varanus albigularis* – egg meal (Secor and Phillips, 1997); 12, *Varanus albigularis* – turkey/snail meal (Secor and Phillips, 1997); 13, *Varanus albigularis* – rodent meal (Secor and Phillips, 1997).

largely due to the differences in SDA given the similarities in meal energy. For the Gila monster, this relationship between meal energy and SDA is similar to that of other lizards with respect to meal energy. Plotting SDA against meal energy for 11 species of lizards ranging in mass from 5 to 8100 g revealed a linear relationship, with SDA equaling on average 17.5±2.1% of meal energy (Fig. 9).

Stomach clearance

Within the first 24 h after feeding, Gila monsters had cleared relatively little (13.8%) of the ingested meal from the stomach (Fig. 2). By day 3, 71.7% of the ingested meals had passed from the stomach, indicating an increase in digestion and passage after the first day. The stomach of the Gila monsters may require a day to upregulate acid production to effectively start breaking down the meal. Alternatively, the slower initial rate of emptying may be due to added time needed to break down the skin and hair of whole prey. Given the rate of meal passage at day 3, we would suspect these Gila monsters to have completely cleared their stomach of the meal within the next 24 to 48 h. In comparison, infrequently feeding and frequently feeding snake species, all digesting rodent meals 25% of their body mass at 30°C, have cleared 15% and 30% of their stomach contents by day 1, and 60% and 90% by day 3 of digestion, respectively (Secor and Diamond, 2000). Apparently, the rate of gastric breakdown of Gila monsters is more similarly matched to those of infrequently feeding compared to frequently feeding snakes.

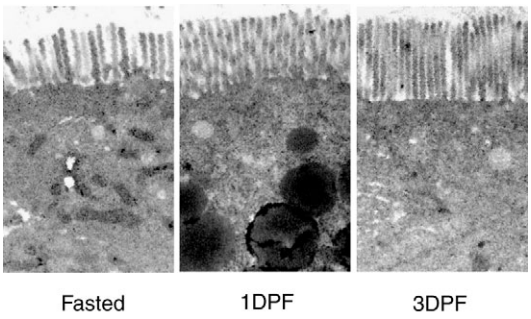

Fig. 10. Transmission electron micrographs of the intestinal brush border membrane of fasted and fed *Heloderma suspectum*. Scale bars, 1 μm. Note the presence of fat droplets within the enterocytes at 1 day postfeeding (1DPF) and the apparent lengthening of microvilli by day 3 of digestion (3DPF).

Small intestinal response to prey ingestion

In our study, feeding failed to stimulate a significant increase in small intestinal mass, although for each feeding group small intestinal mass had increased on average by at least 50% compared to fasted lizards. More modest increases in small intestinal mass with feeding (<50%) have been observed for several species of lizards and actively foraging snakes (Secor and Diamond, 2000; Secor, 2005b; Tracy and Diamond, 2005). Possibly, a larger sample size of Gila monsters may reveal a significant postprandial increase in small intestinal mass. When viewed at the microscopic level, the Gila monster intestine exhibited a postprandial increase in mucosal thickness, largely due to the 68% increase in enterocyte width that generated more than a twofold increase in enterocyte volume. Many of the enterocytes of fed lizards possess large vacuoles filled with lipids (Fig. 10), similar to those observed for fed pythons (Lignot et al., 2005; Ott and Secor, 2007a). A cursory examination of the brush border membrane of the enterocytes revealed a modest increase in microvillus length after feeding (Fig. 10). However, Gila monsters do not experience the four- to fivefold increase in microvillus length previously observed for boas and pythons (Ott and Secor, 2007b).

For the Gila monster there is a distinct gradient in function from the proximal to distal portion of the small intestine. Each of the studied nutrients declined in uptake rates by 31–97% from the proximal to distal segment. Similar positional gradients in intestinal function have been observed for fishes, amphibians, other reptiles, birds and mammals, and reflect a distal decrease in nutrient transporters owning to the progressive decline in luminal nutrients within the intestine (Karasov et al., 1985; Karasov et al., 1986; Buddington and Hilton, 1987; Ferraris et al., 1989; Buddington et al., 1991; Secor and Diamond, 2000; Secor, 2005a).

The Gila monster's small intestine upregulated the active uptake of D-glucose, but did not experience similar upregulation in the uptake of the two amino acids studied. With feeding, D-glucose uptake increased for both the proximal and middle intestinal segments, whereas the distal segment exhibited practically no measurable D-glucose uptake for either fasted for fed lizards. Although the small intestine lacked a detectable increase in amino acid uptake, it did experience a postprandial

increase in APN activity (measured only for the proximal segment). Such an increase in the activity of this peptide hydrolase is expected given the high-protein content of the Gila monster's natural diet. While it is predicted that feeding would trigger the matched upregulation of both APN activity and amino acid transport, similar disassociation in postprandial response (APN activity upregulated and amino acid transport unchanged) has been observed for the blood python *Python brongersmai* (Ott and Secor, 2007a).

As a product of small intestinal mass and mass-specific rates of nutrient uptake, the total intestinal uptake capacity of each of the studied nutrients increased significantly with feeding. The 1.7- to 3.3-fold increase in nutrient uptake capacity is on average contributed to equally by the postfeeding difference in intestinal mass and nutrient uptake rates.

Exendin-4 and digestion events

One of the goals of this study was to assess the potential role of exendin-4 in the regulation of the Gila monster's postprandial metabolic and intestinal responses. We know that plasma concentration of exendin-4 increases rapidly after feeding, peaking within 2 h before decreasing to prefeeding levels within 24 h (Christel and DeNardo, 2006). Hypothetically, exendin-4 exerts its regulatory effect within the first 24 h after feeding on metabolism, intestinal structure, and/or intestinal function. The comparison of postprandial metabolism of lizards that were force fed rodents under anesthesia or ate them voluntarily failed to reveal a significant impact of exendin-4 levels on the peak, duration and overall magnitude (SDA) of the metabolic response. Likewise, we did not find any differences between these two treatments one day after feeding in intestinal mass and length, in mucosal and serosal thickness, or in enterocyte width, length or volume. The presence (or absence) of circulating exendin-4 similarly did not significantly affect nutrient uptake rates, although as seen in Fig. 6, proximal and middle uptake rates of the two amino acids averaged 39% greater from lizards with circulating exendin-4 compared to lizards lacking exendin-4 release. Interestingly, Gila monsters without circulating exendin-4 (1DPF-anes) exhibited significantly greater activity of APN from the proximal intestine compared to normally fed lizards at 1 or 3 days postfeeding. Given the lack of treatment differences in intestinal mass or nutrient uptake, nutrient uptake capacity was not affected by the lack of circulating exendin-4. Therefore we conclude that exendin-4 has no more than a marginal role in the postfeeding metabolic and intestinal responses of the Gila monster. It is possible that exendin-4 would have a detectable impact on gut morphology and function if larger meals were fed, as Gila monsters are known to ingest meals up to threefold greater than we used. However, meal sizes similar to that used in this study stimulate a dramatic increase in endogenous exendin-4 release and thus we would expect a detectable response if the metrics of gut morphology and function that we used were targets of exendin-4 (Christel and DeNardo, 2006). These findings do not preclude exendin-4 from being involved in the signaling pathways of other digestive responses. Similar examinations of other components of digestion (e.g. gastric acid and enzyme activity, pancreatic peptidase production, additional intestinal hydrolase activity) may reveal the regulatory role of exendin-4 in the digestive process.

Adaptive significance

Gila monsters are strict nest predators that eat neonatal mammals and the eggs of reptiles and birds (Beck, 2005). Given the patchiness and seasonality of these resources, Gila monsters travel long distances in the search of food, but forage relatively infrequently, spending up to 95% of their time resting within shelters (Beck, 2005). While best described as an active forager, Gila monsters feed much less frequently than other active foraging lizards. Hence, combined with their ability to consume large meals (>30% of their body mass), Gila monsters possess a feeding habit more similar to that of sit-and-wait foraging snakes. Additionally, Gila monsters possess relatively low standard metabolic rates that are 50% of that predicted for a similar size lizard (Beck and Lowe, 1994). Given these features of their basal metabolism and feeding habits, we predicted that Gila monsters would exhibit regulatory responses of their digestive system similar to those observed for infrequently feeding, sit-and-wait foraging snakes, which is characterized by wide regulation of intestinal performance with feeding and fasting.

The wide range of regulatory responses to feeding and fasting exhibited by animals can be viewed as a continuum: at one end are frequently feeding species that experience no significant regulation of intestinal performance, whereas at the other end of the continuum are species that experience long bouts of fasting and regulate intestinal performance dramatically with each meal. Although we expected Gila monsters to fall out near the end with infrequently feeding snakes, they occupied a more intermediate position. Although, they experience a significant postprandial increase in intestinal nutrient uptake capacity, the magnitude of this response is well short of that experienced by infrequently feeding snakes and estivating anurans, which characteristically upregulate intestinal nutrient uptake capacity by 5- to 30-fold following feeding (Secor and Diamond, 2000; Secor, 2005b; Ott and Secor, 2007a). The larger regulatory responses of these snakes and anurans are the result of a doubling to tripling of small intestinal mass and a three- to tenfold increase in mass-specific rates of nutrient uptake. The more modest response we observed for Gila monsters places them closer to the range of responses observed for more frequently feeding amphibians and reptiles (Secor, 2005a). In general, these animals experience less than a doubling of the intestine's capacity for nutrient uptake with feeding. But unlike Gila monsters, none of these species significantly upregulate intestinal uptake capacity for all studied nutrients (Secor, 2005a). Hence, the adaptive interplay between the Gila monsters' feeding ecology and digestive physiology may reflect an intermediate suite of selective pressures that has resulted in their more modest regulation of digestive performance.

List of abbreviations

APN	aminopeptidase-N
GI	gastrointestinal
LNA	leucyl-β-naphthylamide
RER	respiratory exchange ratio
RM-ANOVA	repeated-measures analysis of variance

RQ	respiratory quotient
SDA	specific dynamic action
SMR	standard metabolic rate
$\dot{V}_{CO_2}$	rate of carbon dioxide production
$\dot{V}_{O_2}$	rate of oxygen consumption

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