A vertebrate model of extreme physiological regulation

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Investigation of vertebrate regulatory biology is restricted by the modest response amplitudes in mammalian model species that derive from a lifestyle of frequent small meals. By contrast, ambush-hunting snakes eat huge meals after long intervals. In juvenile pythons during feeding, there are large and rapid increases in metabolism and secretion, in the activation of enzymes and transporter proteins, and in tissue growth. These responses enable an economic hypothesis concerning the evolution of regulation to be tested. Combined with other experimental advantages, these features recommend juvenile pythons as the equivalent of a squid axon in vertebrate regulatory biology.

The history of biology illustrates the importance of selecting exceptionally suitable species as models: examples include the contributions made by squid axon, pigeon breast muscle, *Necturus* kidney and *Drosophila* to our understanding of excitable membranes, oxidative metabolism, kidney function and population genetics, respectively. These models distinguished themselves by exaggerated structures or responses, or by experimental convenience with respect to the phenomenon under study. Once biologists had unravelled the phenomenon in the model species, they could devise experiments for doing so in species (such as humans and other mammals) presenting greater experimental difficulties but with more practical importance.

A field now in need of such a model species is vertebrate regulatory biology. Many processes are regulated on various timescales by food intake: rapid responses include release of gastrointestinal hormones, switching-on of gastrointestinal secretions, upregulation of nutrient transporters and hydrolases in the gut, and a rise in metabolic rate to accompany digestion; slow responses to chronically increased food intake (for example, during lactation, accompanying an athletic lifestyle, or for heat production in a cold environment) include growth of the heart, kidney, liver and intestine, and adaptations of pulmonary and cardiac function.

Humans and the usual mammalian model species (rats, mice and rabbits) are adapted to consuming small meals (equivalent to only a few per cent of body mass) frequently (many times daily). Hence the gut usually contains food, and fluctuations in loads upon metabolic processes are modest. As a result, regulatory responses have evolved to encompass only modest factorial spans (Table 1), making them difficult to study experimentally, despite their physiological and clinical importance.

A new vertebrate model that exhibits much larger regulatory responses would be valuable for two reasons. One reason is to advance our understanding of regulatory phenomena, such as organ growth and atrophy or signal pathways for hormone release. The other reason is to understand the evolution of regulation itself. Although many biological parameters are regulated reversibly, others are fixed and unresponsive to changes in conditions. For instance, the masses of the mammalian kidney and intestine vary with food intake, but those of the pancreas or brain do not; likewise, the activity of intestinal sucrase, but not of erythrocyte enzymes, varies with food intake. Could these contrasting outcomes depend on the relative costs of maintaining a component 'ready to go' compared with synthesizing it only when needed, and also depend on the temporal variation in component operation typically required over an animal's adult life? (By analogy, someone driving a car in normal traffic finds it cheapest to keep the car's engine running while stopping briefly at traffic lights, but turns off the engine, thus saving fuel consumption, and restarts it after stopping at a railroad while waiting for a long train to pass.) Testing this evolutionary hypothesis requires a system in which the relative costs of maintaining compared with periodically synthesizing certain biological machinery can be separately identified and measured.

We sought such a model among animal species that, unlike rats and humans, are adapted to consuming large meals at long, erratic intervals. Examples of such species include lions, wolves and some deep-sea fishes. As experimentally more tractable candidates, we tested eighteen species of frogs, lizards, turtles and snakes that were reputed to consume large meals. We measured maximum voluntary meal size and post-feeding increases in metabolic rate, enzyme and transporter activities, and organ masses.

The most promising candidates proved to be snake species that obtain their prey by waiting in ambush. Field studies of such snakes show that the mass of a prey animal, swallowed whole without chewing, averages one-quarter of the snake's body mass but ranges up to 1.6 times the snake's mass[1,2]. (That is analogous to a person weighing 62 kg swallowing a 100 kg meal in one gulp.) Typical feeding intervals for such snakes in the wild are one or two months, but may exceed one year[1,3]. Our eventual choice of species was the Burmese python, *Python molurus*. Adults are among the largest snakes, reaching 6.5 m long and 100 kg , and they consume large mammals (including humans)[3]. However, juveniles weigh only 0.1–1.0 kg, consume rats and mice, are popular pets, are available from commercial breeders, and as experimental animals possess other virtues that we shall describe.

Table 1 Comparison of regulatory spans in pythons and mammals

	Factorial increase	
Post-feeding response	Pythons	Mammals
Kidney mass	2.1	1.1 (m)
Intestinal mucosal mass	2.2	1.6 (m)
Plasma glucose	2.3	1.2 (h)
Plasma free fatty acids	2.5	1.5 (h)
Intestinal maltase activity	3.0	1.3 (r)
Intestinal peptidase activity	5.0	1.8 (r)
Intestinal microvillus length	6.0	1.6 (ha)
Intestinal amino acid transport rates	10	2.0 (m)
Intestinal glucose transport rates	41	1.7 (m)
Plasma insulin	41	5.0 (h)
Metabolic rate	44	1.5 (h)
Plasma cholecystokinin	52	6.5 (h)
Plasma triglycerides	160	1.7 (h)

Numbers are post-feeding regulatory spans of various quantities: that is, the factorial increase from fasting levels to peak levels after feeding. For instance, plasma triglyceride levels rise by a factor of 160 in pythons but by only 1.7 times in humans. Note that all regulatory spans are much greater in pythons than in well studied mammal species (m, mice; ha, hamsters; r, rat; h, humans). Sources: for pythons, refs 4 and 8 and personal observations; for mammals, published references available upon request.

Regulatory responses to feeding

Upon swallowing its prey, a python coils up and remains nearly motionless (except for breathing deeply) throughout the 5–11 days required to complete digestion (larger meals requiring more days). This apparent inactivity conceals vigorous internal metabolic activity. Table 1 summarizes factorial magnitudes of some underlying regulatory responses and compares them with the much smaller responses of humans or rodents. These responses all revert to fasting levels by the time of defecation.

A python's metabolic rate, as measured by rates of oxygen consumption ($\dot{V}_{O_2}$), rises within 3 h of feeding, peaks at 1–2 days, and declines to fasting levels at 4–16 days (Fig. 1). Peaks in $\dot{V}_{O_2}$ are up to 44-times fasting $\dot{V}_{O_2}$ in snakes digesting meals equal to the snake's own body mass[4] (Fig. 1). For comparison, the highest factorial post-feeding $\dot{V}_{O_2}$ rise reported for any digesting mammal is 2.0, for dogs[5]. The highest factorial $\dot{V}_{O_2}$ peak reported for a mammal under any conditions is 45 for a galloping racehorse, which is virtually the same as for a digesting python. However, horses can sustain a gallop for only a few minutes, whereas digesting pythons maintain elevated $\dot{V}_{O_2}$ for up to 2 weeks. Pythons' high factorial peak $\dot{V}_{O_2}$ is due not only to their high absolute $\dot{V}_{O_2}$ during digestion, but also to their low standard (fasting) metabolic rate, which is about 13 times lower than that of a similarly sized mammal at the same body temperature[6].

Pythons' increased $\dot{V}_{O_2}$ during digestion arises from metabolic costs of regulatory processes such as switching-on gastrointestinal secretions, upregulating enzymes and transporters, and stimulating rapid growth of organs. The secretion of hydrochloric acid by the stomach causes gastric pH to plummet from 7 to 1 within one day, and to remain low for one week (compared with only a few hours in humans). In the intestinal brush border, the activities of the enzyme amino-oligopeptidase and of the glucose and amino-acid transporters rise by a factor of 5–40 within 1–3 days[7,8]. The small intestine doubles in wet and dry mass within 1 day, largely as a result of a sixfold increase in microvillus length and a doubling of mucosal enterocyte volume (Fig. 2). Within 1–3 days, there are also 50–100% increases in masses of the stomach, liver, pancreas, heart, lungs and kidneys (Fig. 3).

While growth of the small intestine, stomach, liver and pancreas is obviously related to their role in digestion, it is initially surprising that the heart, lungs and kidneys grow as well. However, these organs too experience increased work loads during digestion. Increased O_2 consumption (Fig. 1) and CO_2 exhalation require heart rate, blood flow and ventilation to increase by 3–5 times (S.M.S., J. Hicks and A. Bennett, unpublished observations), whereas increased production of metabolic waste requires increased renal excretion. All of these organs atrophy back to fasting levels after defecation.

Organ growth and enzyme and transporter synthesis require biosynthetic energy. Yet they are underway or have peaked at one day after feeding, when the swallowed rat is still largely intact within the snake's stomach and when intestinal digestion and absorption have scarcely begun. Evidently, energy and substrates for growth and synthesis must initially be mobilized from the snake's stored energy reserves, not from the rat's body. This mobilization is reflected within a day in a 160-fold rise of plasma triglycerides, possibly originating from the large, paired fat bodies within the python's body cavity and causing the plasma at day 1 to change colour from clear to milky-white. That is, digesting pythons operate on the principle of many North American self-service petrol stations: pay before pumping.

The evolution of regulation

The high $\dot{V}_{O_2}$ of digesting pythons reflects their high costs of digestion. In part, these high costs are due to pythons' exceptionally large meals: their total cost of digestion (also known as specific dynamic action (SDA)), which is calculated as $\dot{V}_{O_2}$ beyond the standard metabolic rate and integrated over the period of digestion, increases linearly with meal size[4]. However, their relative cost of digestion is also high for any meal size: it represents on average 32% of the ingested meal's energy equivalent[4]. This percentage, termed the SDA coefficient, is only 9% for humans and falls between 4% and 17% for most vertebrate species[4,9]. That is, digesting pythons burn up a much higher fraction of their meal's energy-equivalent content than do humans.

This high relative cost of digestion is surely related to the biosynthetic start-up costs that digesting pythons incur in synthesizing organs and proteins that have atrophied or been repressed during fasting. Humans and other frequent feeders are spared the start-up costs because they maintain those organs and proteins constantly. Conversely, pythons spare themselves much of the high maintenance cost of those organs and proteins incurred in frequent feeders, by letting them regress after digestion is complete. For instance, the small intestine, heart, liver and kidneys are among the organs with the highest metabolic rates, so that they contribute to only one-eighth of a rat's body mass, but one-quarter to its basal metabolism[10].

Did pythons evolve these extreme feeding-related regulatory responses because of their very infrequent meals? That is, are energy budgets that include the occasional high start-up costs associated with gut upregulation lower than energy budgets that include high everyday maintenance costs such as would be incurred

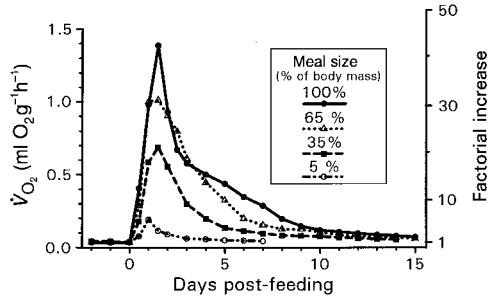

Figure 1 Oxygen consumption rates (left ordinate: $\dot{V}_{O_2}$ in units of ml g^{-1} h^{-1}) of juvenile Burmese pythons before and after consuming rodent meals equivalent to 5, 35, 65 and 100% of the snake's body mass. The right ordinate replots values as multiples of the fasting metabolic rate measured before feeding. Note that $\dot{V}_{O_2}$ rises steeply to a peak within 1–2 d of feeding, and then declines back to fasting levels within 4–16 d; and that larger meals elicit bigger and longer responses. For comparison, the factorial increase in $\dot{V}_{O_2}$ is up to 1.5 in a digesting human, 18 in a running human, and 45 in a galloping racehorse. Data are from ref. 4.

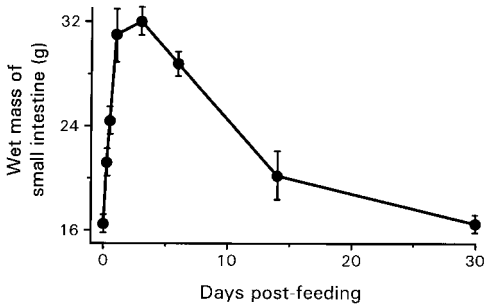

Figure 2 Wet mass of small intestine of pythons as a function of time after consuming rodent meals equivalent to 25% of snake body mass. Note that intestinal mass doubles within 24 h; dry mass yields essentially the same results. Data are from ref. 7.

by maintaining organs and proteins needlessly in readiness during pythons' characteristic long fasts? This qualitative reasoning, based on trade-offs of costs, exemplifies the general economic hypothesis regarding the evolution of regulation that we formulated earlier. The challenge in evaluating this qualitative hypothesis is to test it quantitatively, by actually measuring the costs being traded off against each other.

Snakes lend themselves to such a quantitative test. Many snake species besides pythons share pythons' feeding habits: expending little energy in hunting, waiting motionlessly to ambush prey, and then consuming large prey at infrequent intervals. But other snake species feed like humans and rats: they actively search for prey and consume small meals at frequent intervals. Hence we measured metabolic costs and regulatory spans of digestion in four species of infrequently feeding, ambush-hunting snakes and in four species of frequently feeding, actively foraging snakes, to all of which we fed rodent meals equivalent to 25% of the snake's body mass.

As summarized in Fig. 4, we found a clear physiological dichotomy between the two groups of snakes. (Phylogenetic independent-contrast analysis[11,12] demonstrates that the dichotomy is indeed related to feeding habits and not to phylogenetic history.) Frequent feeders exceed infrequent feeders in standard metabolic rate by an average factor of 2.1. However, infrequent feeders exceed frequent feeders by a factor of 2.0 in relative cost of digestion (SDA coefficient); the coefficient's values of only 14–15% for frequently feeding snakes (much lower than the values of 21–35% for infrequent feeders) are similar to values for frequently feeding mammal, bird and invertebrate species[4]. Infrequent feeders also exceed frequent feeders by a factor of 2.4 for the factorial increase in $\dot{V}_{O_2}$ upon feeding. In none of the four frequent feeders does the intestinal brush-border uptake rate of any of the five studied solutes, nor the wet or dry mass of any of the eight studied internal organs, increase significantly on feeding. In all four infrequent feeders, however, the uptake rates of all five solutes are upregulated upon feeding (by factors of up to 28), as are the masses of the small intestine and liver in all four species and of the stomach, kidneys and pancreas in some species. That is, even if the attention is confined to snakes, infrequent feeders save on maintenance costs, as reflected in their lower standard metabolic rates (because they do not keep organs and proteins ready for the next meal), but they thereby incur larger start-up costs and require high regulatory spans with each meal.

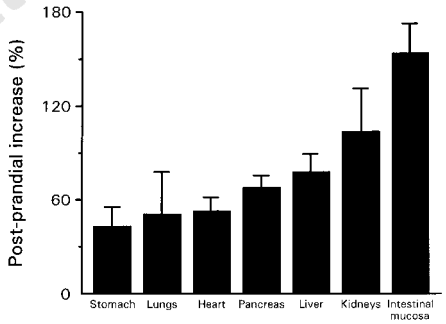

Figure 3 Percentage increase (as percentage of fasted mass) in the wet mass of python organs at 1 or 3 d after consuming rodent meals equivalent to 25 or 65% of snake body mass. Organ dry masses yield essentially the same results. Note that all organs increase rapidly in mass upon feeding, by at least 50%. Data from ref. 8.

Of these eight species of snake, the two for which we have the most information about feeding habits in the wild are the frequently feeding coachwhip (*Masticophis flagellum*) and the infrequently feeding sidewinder (*Crotalus cerastes*)[1,13]. The former consumes meals averaging 15% of its body mass at 10-day intervals; the latter consumes meals averaging 25% of its body mass at 6-week intervals. Using physiological parameters that we measured for each species—standard metabolic rate and costs of digestion (SDA)—we calculated the sum of these two energy costs as a function of meal interval for each species. As illustrated in Fig. 5, the combined costs, which contribute to a large fraction of the snakes' energy budgets in the wild[1], cross over at a feeding interval of 4 weeks. At intervals of less than 4 weeks, a snake with sidewinder-like physiology (that is, large regulatory responses and low standard metabolic rate[14]) is thereby obliged to expend more energy (because it would often incur its high start-up costs), but it would incur lower expenditure for intervals longer than 4 weeks (because it saves on the coachwhip's high maintenance costs and rarely incurs its own high start-up costs). This calculation agrees with field observations: the coachwhip has co-evolved its physiology with a 10-day natural feeding interval, whereas the sidewinder's physiology has co-evolved with a 6-week natural feeding interval.

Thus, Fig. 5 confirms quantitatively for snake metabolic physiology the hypothesis that cost trade-offs caused regulation to evolve for some biological processes but not for others.

Outlook

We conclude by mentioning three fruitful directions for future research.

Cost trade-offs underlying the evolution of other regulatory systems. Figure 5 may serve as a prototype for calculations comparing

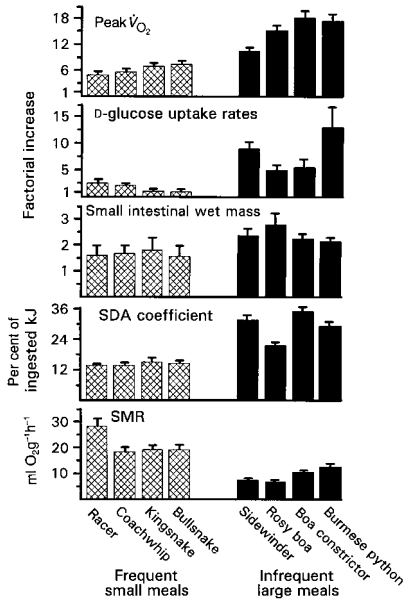

Figure 4 Factorial increases (relative to values for fasting individuals of the same species) of five parameters in four frequently feeding (left) and four infrequently feeding (right) snake species. The parameters are: whole-animal peak O_2 consumption rate ($\dot{V}_{O_2}$), uptake rate by the small intestinal brush-border of D-glucose, small intestinal wet mass at 1 d post-feeding, and relative cost of digestion (SDA coefficient), all measured during digestion of a meal equivalent to 25% of the snake's body mass; and fasting metabolic rate (equivalent to standard metabolic rate or SMR, allometrically corrected to a body mass of 350 g). Note that the species that habitually consume infrequent large meals exhibit much bigger responses to feeding than do the species that habitually consume frequent small meals, even though all eight species were studied after consuming the same size of meal.

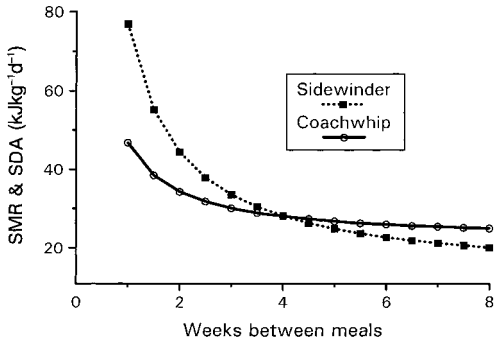

Figure 5 Time-averaged daily partial energy budgets as a function of interval between meals, calculated for two snake species that differ in physiology and in feeding ecology. Wild sidewinders capture prey that average 25% of their body mass at average intervals of 6 weeks. Wild coachwhips capture prey that average 15% of their body mass at intervals of 10 d. Energy budgets are calculated from fasting metabolic rates (SMR, standard metabolic rate) and costs of digestion (SDA) measured for each species, using average meal sizes in the wild but using various feeding intervals ranging from 1 to 8 weeks. Fasting metabolic rate is 2.1-fold higher in coachwhips, but the cost of digestion for equivalent meal sizes is 2.2-fold higher in sidewinders. Note that sidewinders have more a costly energy budget at feeding intervals less than 4 weeks but have a less costly budget at feeding intervals greater than 4 weeks.

the costs of other regulated and non-regulated systems, as a function of interval between demands on the system. Does this simple evolutionary reasoning account for why regulation has or has not evolved in other cases? In snakes, we could calculate the costs of digestion and of maintaining the underlying metabolic machinery from the whole snake's $\dot{V}_{O_2}$, because digestion and its machinery account for much of the snake's energy budget (20–40%)[1]. In the case of regulated systems accounting for only a small fraction of the animal's energy budget, our proposed test will require somehow measuring or calculating in isolation the relative costs of upregulating and of maintaining the system. For instance, one could compare the cost of maintaining a high activity of a regulated protein, given constant protein turnover, with the cost of maintaining a low activity combined with infrequent bouts of increased synthesis of the same protein if it were regulated.

Other big eaters. By standards familiar to us (based on humans and rats), the metabolic physiology of ambush-hunting snakes is exceptional. Actually, many other animal species resemble them in alternating large meals with long fasts. These other species should also be examined for the possible existence of extreme metabolic regulation. The long list of candidate species includes lions, wolves, Komodo dragons and some abyssal fishes, which all consume huge meals; hibernating animals; birds and whales that migrate long distances without feeding; young seals, penguins and petrels that fast for several months after weaning or fledging before going to sea to feed; and adult penguins and albatrosses that fast for several months during courtship and egg incubation.

Pythons as model species in vertebrate regulatory biology. The large regulatory responses of pythons compared with humans and rats (Table 1) recommend them as a model species. In addition, they offer other advantages besides that quantitative one. Being vertebrates, their proteins possess much higher sequence homology with mammalian proteins than do squid and *Drosophila* proteins. The python's linear anatomy is convenient for surgical intervention, such as pancreatectomy or intestinal resection and reanastomosis. Contrary to the public image of pythons as being vicious and dangerous, juvenile pythons are docile and much less likely to bite than are rats, and are popular pets for children. They are cooperative experimental subjects, for instance in tolerating a swallowed intragastric pH electrode for one week. They are commercially available through reptile breeders, are not an endangered species subject to legal restrictions, and are far cheaper, easier and cleaner to house, maintain and feed than are similarly sized mammals (only two meals and two combined semi-solid defecations/urinations per month). Their clutches of up to 100 eggs permit reducing inter-individual genetic variation by using siblings. They do not arouse the controversy associated with medical research on similarly sized mammals.

As examples of the potential research value of pythons, listed below are five projects that are currently proving profitable. (1) Molecular mechanisms of regulation of the glucose transporter SGLT1 are being studied in pythons over a much greater regulatory span (40-fold) than in rats (twofold) (M. Martín, E. Wright and S.M.S., unpublished observations). (2) Conlon *et al.*[15,16] have isolated and sequenced eight python gastrointestinal hormones for synthesis and infusion to determine their physiological actions, and for developing assays to measure physiological release and plasma levels. Post-feeding surges in plasma levels prove to be high (for example, they increase by a factor of 52 for python cholecystokinin). Python insulin has an amino-acid sequence otherwise undocumented in nature, but previously developed by synthesis as a superactive analogue of human insulin[15]. (3) Surgically modified pythons with bypassed pancreating and biliary ducts, a balloon catheter in the stomach, surgically isolated intestinal loops, and chronically implanted intestinal catheters for infusing nutrient solutions, lend themselves to experiments designed to unravel neurohormonal regulatory signals. (4) The large and rapid organ growth that occurs in pythons within one day of feeding (Figs 2, 3) makes them convenient models for studying intestinal and kidney hypertrophy, and unique models for studying physiological pancreatic and cardiac hypertrophy (for which we have no mammalian model). (5) The very large increases in whole-animal $\dot{V}_{O_2}$, ventilation, heart rate, and blood flow of fed pythons make them convenient for studying cardiopulmonary regulation.

These examples illustrate the potential of juvenile pythons to become the equivalent of the squid axon in vertebrate regulatory biology. □

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