

VENTILATORY AND CARDIOVASCULAR RESPONSES OF A PYTHON (*PYTHON MOLURUS*) TO EXERCISE AND DIGESTION

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Accepted 26 May; published on WWW 20 July 2000

Summary

To investigate the potential limiting steps of peak metabolic rates, we examined gas exchange rates (\dot{V}_{O_2} , \dot{V}_{CO_2}), respiratory exchange ratio (RER), breathing frequency, tidal volume, minute ventilation volume (\dot{V}_E) as well as the heart rate, systemic blood flow and stroke volume of Burmese pythons (*Python molurus*) while fasting at rest, exercising, digesting and exercising while digesting. All measured variables increased significantly during exercise (crawling at 0.4 km h^{-1} and at $\dot{V}_{O_2\text{max}}$), highlighted by a 17-fold increase in \dot{V}_{CO_2} and a 24-fold increase in \dot{V}_E . During the digestion of a meal equivalent to 25% of the snake's body mass, pythons responded with increases in \dot{V}_{O_2} and heart rate similar to those experienced during

exercise, along with a 4.5-fold increase in systemic blood flow. Interestingly, pythons hyperventilated while exercising, whereas they hypoventilated during digestion. The combined demands of exercise and digestion resulted in significantly higher \dot{V}_{O_2} , \dot{V}_{CO_2} , breathing frequency and heart rate than during either exercise or digestion alone. Evidently, the capacities of the ventilatory and cardiovascular systems to transport oxygen to locomotor muscles are not a limiting factor in the attainment of peak metabolic rates during exercise in pythons

Key words: reptile, snake, *Python molurus*, exercise, digestion, gas exchange, ventilation, cardiac output, specific dynamic action.

Introduction

Elevations in energy expenditure are accompanied by increments in both ventilation and cardiac output to maintain appropriate levels of gas exchange. These responses are usually assumed to be maximized only during strenuous bouts of locomotion, i.e. running, crawling, flying or swimming (Brett, 1972; Seeherman et al., 1981; Walton et al., 1990; Suarez, 1992) or during other activities involving vigorous contraction of skeletal muscle (e.g. calling by anurans; Taigen and Wells, 1985). In such cases of extreme physical exertion, the elevated cardiac output is largely devoted to skeletal muscle in order to sustain aerobic metabolism. In contrast, it has been demonstrated that Burmese pythons (*Python molurus*) attain maximum rates of oxygen consumption not during strenuous exercise but instead during digestion (Secor and Diamond, 1995, 1997). The increase in metabolic rate generated from the mechanical and physiological processes of digestion is referred to as 'specific dynamic action' (SDA) and represents the energetic cost of processing, digesting and absorbing food (Brody, 1945; Klieber, 1975). For Burmese pythons, SDA can stimulate an increase in oxygen consumption of as much as a 44-fold, an increase that far surpasses the seven- to tenfold increment they experience during vigorous crawling (Secor and Diamond, 1995) or while rapidly contracting their axial

musculature to generate heat during egg incubation (Vinegar et al., 1970).

Considerable attention has been focused on the potential limiting steps in the oxygen transport and utilization cascade. Possible limitations include the capacity of the ventilatory system to take up oxygen, the capacity of the cardiovascular system to deliver oxygen to muscle cells and the oxidative capacity of muscle mitochondria (Taylor et al., 1981; Suarez, 1996; Wagner, 1996). Because pythons experience significant increases in metabolic rate during both movement and digestion, they provide an exemplary model in which to examine and compare potential limits in these two different elevated metabolic states. Both activity and digestion share the same ventilatory and cardiovascular pathways of oxygen intake and transport, but differ in the pathways of oxygen utilization (skeletal muscle *versus* gut tissues). What limits metabolism during these two conditions?

The study of these two metabolic demands, both singly and in combination, may illuminate the specific mechanisms that establish the upper limit to $\dot{V}_{O_2\text{max}}$ during exercise and digestion. For instance, if oxygen utilization by skeletal muscle actually sets the limit to aerobic metabolic rate during exercise, the true functional capacity of other components (ventilatory and cardiovascular) may be revealed in studies on digesting

and/or exercising pythons. The goals of this study were (i) to evaluate for Burmese pythons the increase in gas exchange and cardiopulmonary performance during strenuous exercise alone, (ii) to document the time course and magnitude of these variables in pythons digesting at rest, and (iii) to assess the responses of these variables in pythons exercising while digesting.

Materials and methods

Experimental animals

We used six juvenile (2-year-old) Burmese pythons (*Python molurus* L.) with an average mass of 1380 ± 60 g (mean \pm S.E.M.; range 1160–1600 g). All snakes were purchased as hatchlings from a commercial breeder (Captive Bred Reptiles, Oklahoma City, OK, USA) and maintained at UCLA prior to their transportation and study at UC Irvine. Pythons were housed individually in plastic cages under a light:dark photoperiod of 12h:12h, provided with water weekly, and fed laboratory rats once every 2 weeks. Prior to this study, snakes were fasted for 1 month to ensure that they became post-digestive (digestion is usually completed within 14 days after feeding; Secor and Diamond, 1995). Python care and research were carried out under UCLA Animal Research Committee Protocol Number 93-204 and UCI Animal Research Committee Protocol Number 96-1549.

Blood flow probes and mask attachment

To measure gas exchange, ventilation and cardiac performance simultaneously, we surgically implanted perivascular flow probes (model 2R, Transonic Systems Inc., Ithaca, NY, USA) around the right and left systemic arteries of each snake and attached a sealed plastic mask around its head. For the surgery, we initially anesthetized each snake by placing it within a sealed 4l container containing a cloth soaked with halothane (Halocarbon Laboratories, River Edge, NJ, USA). Once a snake became completely anesthetized (flaccid body tone), we laid it on its dorsum and scrubbed the ventral and lateral scales in the region of the heart (determined by observing the heart palpating against the ventral scales) with topical antiseptic (Betadine solution, Purdue Frederick Co., Norwalk, CT, USA). To maintain anesthesia during surgery, we continuously administered halothane using a vaporizer (2–3 % halothane mixture) attached to a mask held around the snake's head. We then made an 8cm incision several centimeters distal from the heart between the ventral and first row of dorsal scales on the snake's right side. The incision was retracted, and the right and left systemic vessels were located and isolated. We attached a flow probe to each vessel and passed their wire leads through a small incision made through the body wall and skin approximately 10cm distal to the flow probes. The leads and their terminal connectors were sutured to the dorsal scales at several locations to minimize any snagging or twisting of the leads. We closed the incisions with an inner (muscular layer) and outer (scales) set of interrupted sutures (3-0 Vicryl, Ethicon Inc., Somerville, NJ, USA) and a

thin application of cyanoacrylate tissue glue (Nexaband, S/C-TriPoint Medical, Raleigh, NC, USA) to the outer wound. Following surgery, each snake was administered intramuscularly 0.5 mg kg^{-1} of an analgesic (Flunixin meglamine, Phoenix Pharmaceutical Inc., St Joseph, MO, USA) and 3 mg kg^{-1} of an antibiotic (Baytril, Bayer Corporation, Shawnee Mission, KS, USA). Snakes typically recovered from anesthesia within 1–2 h.

On the day following surgery, we attached a lightweight plastic mask (mass 6.8 ± 0.2 g, volume 63 ± 1 ml; means \pm S.E.M., $N=6$) around the head of each snake. Each mask was fashioned from thin (0.2 mm thick) pieces of clear plastic and had two ports attached to the dorsal surface of the mask for connecting with incurrent and excurrent air lines. We used 5 min epoxy resin to seal the seams of the masks and to attach the mask to the snake's head. Enough epoxy resin was inserted between the scales and the back edge of the mask to form an airtight seal. We tested each seal by attaching air hoses to the ports, submerging the snake's head under water in a bucket and forcing air into the masks from one of the air hoses. If air leaked out from the seams or edge of the mask (noted by emerging bubbles), it was coated with another application of epoxy. All masks were eventually found to be airtight. Following the attachment of masks, snakes were allowed to rest for 1 day prior to measurements.

Measurements of gas exchange, ventilation and cardiovascular performance

We measured the following seven variables: the rate of oxygen consumption (\dot{V}_{O_2} as $\text{ml kg}^{-1} \text{ min}^{-1}$), the rate of carbon dioxide production (\dot{V}_{CO_2} as $\text{ml kg}^{-1} \text{ min}^{-1}$), breathing frequency (breaths min^{-1}), tidal volume (ml kg^{-1}), minute ventilation volume (\dot{V}_E as $\text{ml kg}^{-1} \text{ min}^{-1}$), heart rate (beats min^{-1}) and blood flow through the right and left systemic arteries ($\text{ml kg}^{-1} \text{ min}^{-1}$). Air lines were attached to the ports of the mask, and we pumped air through the mask at 500 ml min^{-1} . Changes in airflow representing inhalation and exhalation volumes were measured using a calibrated pneumotachograph (8421, series 0-5 LPM, H. Rudolph, Inc., MO, USA) connected to a differential pressure transducer (Validyne MP 45-1-871). A subsample of the excurrent air was continuously pulled through an oxygen analyzer (S-3A, Applied Electrochemistry, Pittsburgh, PE, USA) and a carbon dioxide analyzer (CD-3A, Applied Electrochemistry, Pittsburgh, PE, USA) in series, to measure O_2 and CO_2 concentrations.

Blood flow through the right and left systemic arteries was measured simultaneously with a dual-channel blood flow meter (model T201, Transonic System Inc., Ithaca, NY, USA). Instantaneous blood flow velocity in either the right or left systemic artery was used to determine heart rate. Signals from the blood flow meter, pressure transducer and gas analyzers were continuously retrieved (50 Hz) and stored by a computer-assisted data-acquisition system (MP100, Biopac System, Inc., Santa Barbara, CA, USA). All measurements were undertaken within a walk-in temperature-controlled chamber maintained at 30 ± 1 °C.

Experimental protocol

We simultaneously recorded each variable from fasting pythons at rest over a 3 day period. Twice daily (morning and afternoon), we continuously recorded variables from each snake as it rested in its cage for a minimum of 2 h. Following the 3 day set of resting measurements, each snake was removed from its cage and placed on a motorized treadmill. We increased tread speed to 0.4 km h^{-1} , at which pythons reached $\dot{V}_{\text{O}_2\text{max}}$ (see Results), and recorded variables while the snake crawled at 0.4 km h^{-1} . These measurements were terminated after the snake had maintained a steady rate of crawling for several minutes. We then removed each snake's mask and returned it to its cage. On the following day, each snake consumed a rat meal equivalent to approximately 25% of the snake's body mass. Within 2 h after each snake had swallowed its rat, we reattached and sealed its mask. For the next 72 h, we measured all variables at 8 h intervals (for a minimum of 1 h) from each snake as it rested quietly digesting its meal. Immediately following the 72 h recordings, we removed each snake from its cage, placed it back on the treadmill and recorded variables as before as each snake was digesting and crawling at 0.4 km h^{-1} .

Statistical analyses

We reviewed the sets of continuously recorded data and selected and averaged five 1 min intervals from segments of stable readings. We quantified each variable using the software program *AcqKnowledge* (Biopac Systems, Inc., Santa Barbara, CA, USA). \dot{V}_{O_2} , \dot{V}_{CO_2} and the tidal volume of single breaths were determined as the area below or above the baseline signal for each gas. The relationships between this area and \dot{V}_{O_2} and \dot{V}_{CO_2} were determined by simulating exhalations with injections of known gas compositions into a sealed mask attached to the air lines. Expired tidal volume was similarly determined from the integrated flow signal from the differential pressure transducer in response to injections of a range of gas volumes into the mask. These calibrations procedures produced very tight correlations between injected gas volumes and integrated flow signal (all $r^2 > 0.98$) and were conducted before, during and after experiments.

We used a repeated-design analysis of variance (ANOVA) to test for a significant treatment effect (time) on post-feeding measurements at rest and separately for significant differences among four sets of data: resting while fasted, crawling while fasted, peak rates while digesting, and crawling while digesting at 72 h post-feeding. In conjunction with ANOVAs, we made some pairwise mean comparisons between sampling periods or sets of data. We also undertook paired and unpaired *t*-tests when appropriate. In the text, we report the results of our analyses in terms of their *P* values. We set $P < 0.05$ as the level of statistical significance and present our results as means ± 1 S.E.M. Sample size (*N*) for all variables was six, unless otherwise noted. All statistical analyses were performed using the microcomputer version of SAS (version 7, SAS Institute, Inc., Cary, NC, USA).

Results

Exercise

On the treadmill, pythons performed lateral undulations to maintain speed. Over the range of measured speeds ($0.1\text{--}0.5 \text{ km h}^{-1}$; not all snakes were measured over the entire range), the available data suggest that \dot{V}_{O_2} increases over the lower speeds and plateaus at around 0.3 km h^{-1} (Fig. 1). We found no significant difference (unpaired *t*-test, $P = 0.36$) in \dot{V}_{O_2} measured at 0.3 and at 0.4 km h^{-1} . Therefore, when crawling at 0.4 km h^{-1} , pythons are apparently experiencing $\dot{V}_{\text{O}_2\text{max}}$. This allows us to compare cardiopulmonary responses of pythons at $\dot{V}_{\text{O}_2\text{max}}$ during exercise with those during digestion.

Gas exchange

\dot{V}_{O_2} and \dot{V}_{CO_2} of exercising fasted pythons increased rapidly to peak at rates 10 and 16.5 times, respectively, resting values (Table 1). During digestion, pythons experienced significant (all $P < 0.001$) increments in both \dot{V}_{O_2} and \dot{V}_{CO_2} during each 8 h interval after feeding until 32 h post-feeding (Fig. 2). Peaks in \dot{V}_{O_2} and \dot{V}_{CO_2} during digestion were attained at 40 h and 32 h post-feeding, respectively, at values 9 and 9.8 times fasting values (Fig. 2). Both \dot{V}_{O_2} and \dot{V}_{CO_2} had declined from peak values by 72 h post-feeding, but still remained significantly above fasting rates. Whereas \dot{V}_{O_2} did not differ between its peak during digestion and that experienced during exercise, \dot{V}_{CO_2} was significantly greater during exercise (Table 1). Therefore, a similar demand for oxygen exchange occurred during these two metabolic states, facilitating comparisons of the patterns of cardiopulmonary support. When digesting pythons exercised, they achieved the highest levels of \dot{V}_{O_2} and \dot{V}_{CO_2} measured, rising to 11.7 and 23.7 times resting values, respectively (Table 1). These levels of gas exchange significantly exceed those observed during either exercise or digestion (Table 1). The post-feeding increment in respiratory exchange ratio (RER, calculated as $\dot{V}_{\text{CO}_2}/\dot{V}_{\text{O}_2}$)

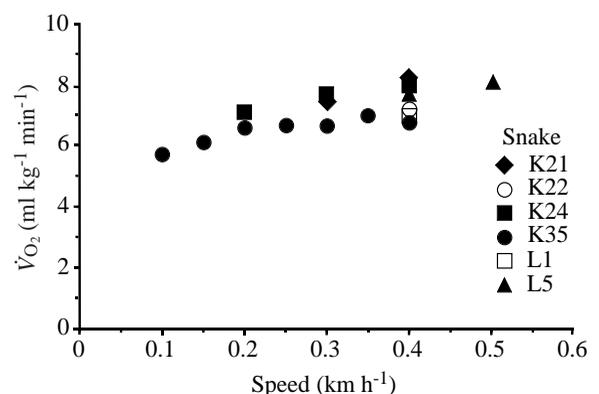


Fig. 1. Rates of oxygen consumption (\dot{V}_{O_2}) as a function of crawling speed for six juvenile *Python molurus* (mass 1380 ± 60 g, mean \pm S.E.M.). Not all pythons were tested at each speed, but all were tested at 0.4 km h^{-1} . Note the apparent plateau of \dot{V}_{O_2} at speeds greater than 0.3 km h^{-1} . We therefore assume that $\dot{V}_{\text{O}_2\text{max}}$ during locomotion is being achieved at 0.4 km h^{-1} .

Table 1. Comparison of gas exchange (\dot{V}_{O_2} , \dot{V}_{CO_2}), respiratory exchange ratio (RER), breathing frequency, tidal volume, minute ventilation (\dot{V}_E), heart rate, blood flow through the right and left systemic arteries, cardiac output (\dot{Q}_{sys}^*), stroke volume (V_s^*), O_2 extraction and CO_2 carriage of juvenile Burmese pythons *Python molurus* (mean mass 1380 ± 60 g) resting while fasted, exercising while fasted, peak during digestion and exercising at 72 h post-feeding

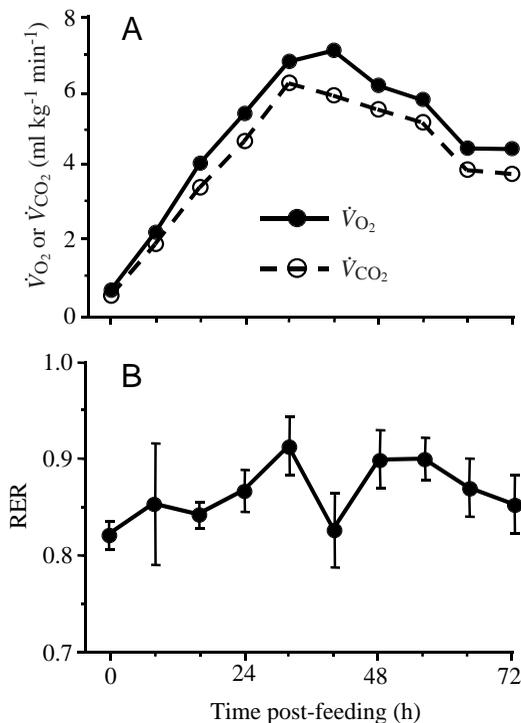
Variable	N	Resting while fasted	Exercising while fasted	Peak during digestion	Exercising at 72 h post-feeding
\dot{V}_{O_2} (ml kg ⁻¹ min ⁻¹)	6	0.76±0.10 ^a	7.6±0.2 ^b	7.2±0.2 ^b	8.9±0.4 ^c
\dot{V}_{CO_2} (ml kg ⁻¹ min ⁻¹)	6	0.62±0.08 ^a	10.3±1.0 ^c	6.7±0.3 ^b	14.7±1.2 ^d
RER ($\dot{V}_{CO_2}/\dot{V}_{O_2}$)	6	0.82±0.01 ^a	1.34±0.10 ^b	0.98±0.01 ^a	1.65±0.14 ^c
Breathing frequency (breaths min ⁻¹)	6	1.8±0.3 ^a	14.7±0.8 ^c	8.9±0.6 ^b	18.0±0.8 ^d
Tidal volume (ml kg ⁻¹)	6	17.4±1.8 ^a	44.1±2.8 ^d	24.3±1.2 ^b	33.0±2.4 ^c
\dot{V}_E (ml kg ⁻¹ min ⁻¹)	6	27.8±2.1 ^a	660±51 ^c	143±12 ^b	587±38 ^c
\dot{V}_E/\dot{V}_{O_2}	6	41.8±9.1 ^b	86.8±5.5 ^d	19.4±1.7 ^a	66.6±5.1 ^c
\dot{V}_E/\dot{V}_{CO_2}	6	50.9±11.4 ^{b,c}	69.4±5.7 ^c	20.2±2.1 ^a	41.9±4.9 ^{ab}
Heart rate (beats min ⁻¹)	6	24.7±1.4 ^a	56.9±1.4 ^b	59.8±0.9 ^b	65.6±1.9 ^c
Left systemic blood flow (ml kg ⁻¹ min ⁻¹)	6	10.8±0.8 ^a	31.8±1.9 ^b	42.9±2.3 ^d	37.4±0.6 ^c
Right systemic blood flow (ml kg ⁻¹ min ⁻¹)	5	7.8±1.9 ^a	21.2±2.5 ^b	43.2±3.4 ^c	24.7±1.0 ^b
\dot{Q}_{sys}^* (ml kg ⁻¹ min ⁻¹)	5	18.9±2.3 ^a	54.9±2.1 ^b	84.5±5.5 ^c	62.0±1.4 ^b
V_s^* (ml kg ⁻¹)	5	0.74±0.06 ^a	0.95±0.04 ^b	1.49±0.10 ^c	0.95±0.03 ^b
O_2 extraction (ml O_2 ml ⁻¹ \dot{Q}_{sys}^*)	5	0.04±0.01 ^a	0.13±0.01 ^c	0.09±0.01 ^b	0.14±0.01 ^c
CO_2 carriage (ml CO_2 ml ⁻¹ \dot{Q}_{sys}^*)	5	0.03±0.01 ^a	0.18±0.02 ^c	0.09±0.01 ^b	0.24±0.02 ^c

Values are presented as means ± 1 S.E.M.; N refers to the number of individual pythons.

For each variable, superscript letters that are shared denote non-significant ($P > 0.05$) differences between means as determined from planned pair-wise comparisons.

Air convection requirements (\dot{V}_E/\dot{V}_{O_2} , \dot{V}_E/\dot{V}_{CO_2}) listed under 'Peak during digestion' were calculated at the time of maximum gas exchange.

was not significant, whereas fasting exercise and digesting exercise each induced a significant (both $P < 0.002$) increase in RER to 1.34 and 1.65, respectively (Table 1).

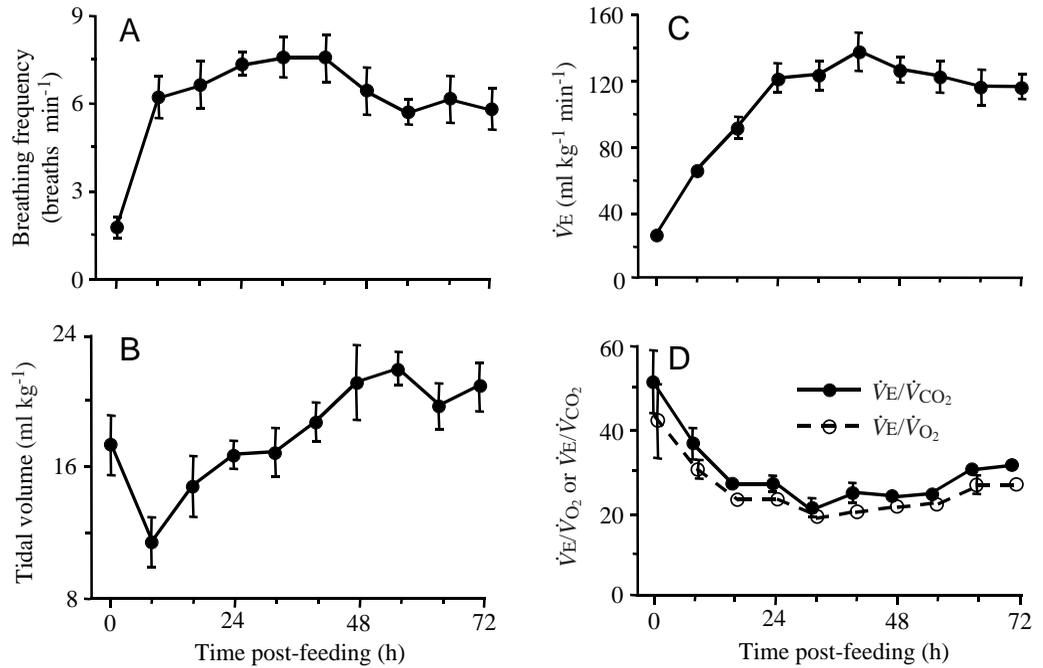


Ventilation

During exercise (fasted), breathing frequency, tidal volume and \dot{V}_E increased by a factor of 8.2-, 2.5- and 24-fold, respectively, over resting values (Table 1). After feeding, all three of these variables also changed significantly (all $P < 0.003$) within 8 h (Fig. 3) compared with resting values. Whereas breathing frequency did not change significantly thereafter for the next 2 days of digestion, \dot{V}_E increased significantly (all $P < 0.001$) during each of the two subsequent 8 h periods. Both breathing frequency and \dot{V}_E peaked at 40 h post-feeding, at rates 4.3 and 5.1 times fasting values, respectively. Tidal volume initially decreased, then returned to fasting levels (16–40 h post-feeding), and later increased to a maximum at 56 h post-feeding (Fig. 3B). By 72 h post-feeding, both breathing frequency and \dot{V}_E had significantly (both $P < 0.027$) declined from the 40 h values. During exercise at 72 h post-feeding, \dot{V}_E increased above peak digestion values and was not significantly different from that during fasting exercise.

Fig. 2. (A) Rates of oxygen consumption (\dot{V}_{O_2}) and carbon dioxide production (\dot{V}_{CO_2}) and (B) respiratory exchange ratio (RER) for six juvenile *Python molurus* prior to (0 h) and up to 72 h after consuming a rat meal equivalent in mass to 25% of the snake's body mass. Vertical bars represent ±1 S.E.M., but bars are omitted if they are smaller than the symbol for the mean value. Note the significant nine- to tenfold increases in \dot{V}_{O_2} and \dot{V}_{CO_2} after feeding and the lack of any significant variation in RER during the first 3 days of digestion.

Fig. 3. (A) Breathing frequency, (B) tidal volume, (C) minute ventilation (\dot{V}_E), and (D) air convection requirements (\dot{V}_E/\dot{V}_{O_2} , \dot{V}_E/\dot{V}_{CO_2}) for six juvenile *Python molurus* prior to (0 h) and up to 72 h after consuming a rat meal equivalent in mass to 25% of the snake's body mass. Vertical bars represent ± 1 S.E.M., but bars are omitted if they are smaller than the symbol for the mean value. Note the significant increases in breathing frequency, tidal volume and \dot{V}_E and the significant declines in air convection requirements after feeding.



Pythons hyperventilated during fasting exercise: air convection requirements more than doubled for \dot{V}_{O_2} (\dot{V}_E/\dot{V}_{O_2}) and increased by 36% for \dot{V}_{CO_2} (\dot{V}_E/\dot{V}_{CO_2}) over resting values (Table 1). During digestion, however, pythons hypoventilated: air convection requirements for \dot{V}_{O_2} and \dot{V}_{CO_2} decreased to levels 46% and 40%, respectively, of rested fasting values (Fig. 3). With exercise, the air convection requirements of digesting snakes compared with resting fasted snakes, increased significantly by 150% and 35%, respectively, for \dot{V}_E/\dot{V}_{O_2} and \dot{V}_E/\dot{V}_{CO_2} , but the levels reached were significantly less than those experienced during fasting exercise (Table 1).

Cardiovascular performance

Resting and fasted pythons responded to exercise and to digestion with more than a doubling of heart rate (Table 1; Fig. 4). When induced to crawl at 72 h post-feeding, heart rate rose to a level that was 2.7 times rested fasting levels and significantly greater than rates attained during either fasting exercise or digestion (Table 1).

During exercise, blood flow through the right and left systemic arteries increased by 172% and 194%, respectively, above resting rates (Table 1). Ingestion induced significant (all $P < 0.0001$) increases in blood flow within 8 h, with rates peaking at 40 h post-feeding at 5.3 and 3.9 times fasting values, respectively, for the right and left vessels (Fig. 4). In response to the joint demands of digestion and exercise, blood flow was actually less than the peaks reached during digestion alone (Table 1).

The combined flow through the right and left systemic arteries provides an approximation of cardiac systemic output (\dot{Q}_{sys}). This underestimates true \dot{Q}_{sys} (and is therefore designated \dot{Q}_{sys}^*) because it does not include the blood entering the carotid, vertebral and coronary vessels that exit the

systemic vessels prior to the positions of the flow probes. We found \dot{Q}_{sys}^* to increase by 2.9-, 4.5- and 3.3-fold above fasted resting values during fasting exercise, digestion and digesting exercise, respectively (Fig. 4; Table 1). Stroke volume (V_s^* , calculated as \dot{Q}_{sys}^* divided by heart rate) increased by 30% during exercise and by 90% during digestion (Table 1; Fig. 4). While exercising and digesting, V_s^* returned to the same level experienced during exercising alone (Table 1).

Discussion

Pythons exhibit a marked increase in cardiopulmonary performance during both exercise and digestion. Although these two activities shared similar rates heart rates and rates of O_2 utilization, they differed in \dot{V}_{CO_2} , RER, ventilation and cardiac output, indicating that patterns of cardiopulmonary support are distinct, even at equal elevated rates of metabolism. In this study, pulmonary performance capacity (i.e. minute volume, \dot{V}_E) was greater during exercise, whereas cardiac performance peaked during digestion. The combined demands of exercise and digestion induced further increases (significantly above values for either exercise or digestion alone) in \dot{V}_{O_2} , \dot{V}_{CO_2} , RER, breathing frequency and heart rate. In the ensuing discussion, we shall discuss the python's response to these sources of metabolic demand separately and in combination.

Cardiopulmonary support during fasted exercise

Pythons responded to exercise with significant increases in gas exchange, ventilation and cardiac output. Crawling at 0.4 km h^{-1} , pythons attained $\dot{V}_{O_{2max}}$, evident from the asymptote in \dot{V}_{O_2} with increasing speed (Fig. 1), a dramatic increase in blood lactate levels (S. Secor and J. Diamond, unpublished data) and the very short duration of their crawling endurance (no more

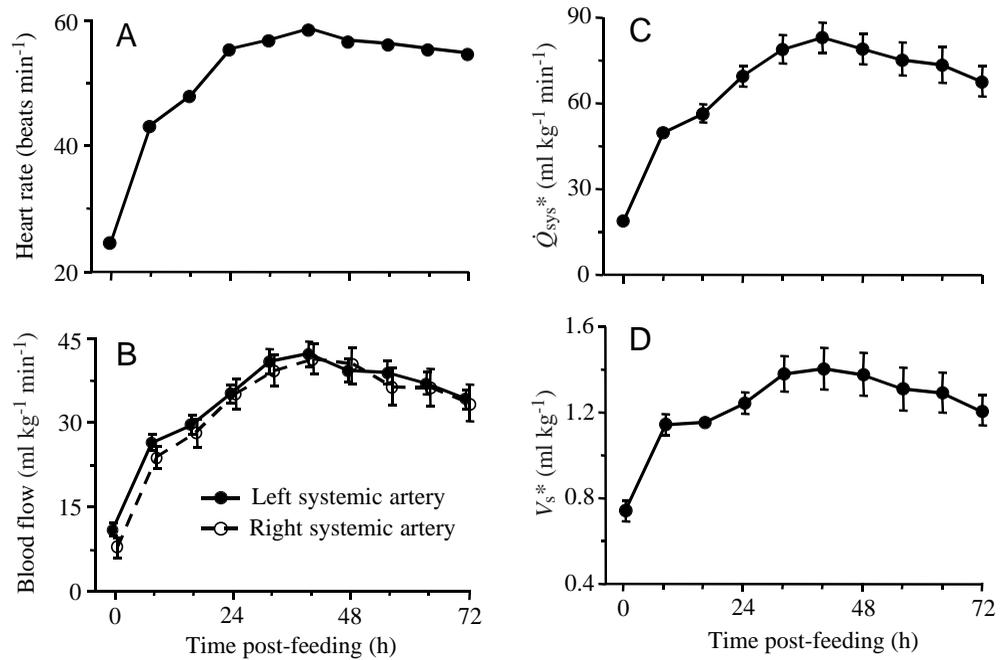


Fig. 4. (A) Heart rate, (B) blood flow through the left and right systemic arteries, (C) systemic cardiac output (\dot{Q}_{sys}^*) and (D) stroke volume (V_s^*) for six juvenile *Python molurus* prior to (0h) and up to 72h after consuming a rat meal equivalent to 25% of the snake's body mass. Vertical bars represent ± 1 S.E.M., but bars are omitted if they are smaller than the symbol for the mean value. Note the significant postprandial increases in heart rate, blood flow, systemic output and stroke volume, all peaking at 40h post-feeding.

than 10 min at that speed). With exercise, \dot{V}_{CO_2} increased more than \dot{V}_{O_2} , resulting in a significant increase in RER that exceeded a value of 1.0, suggesting that exercise induces a state of metabolic acidosis for pythons. The astonishing 24-fold increase in \dot{V}_E during exercise is a product primarily of the 8.2-fold increase in breathing frequency and secondarily the 2.5-fold increase in tidal volume. Joined by the 10-fold increase in \dot{V}_{O_2} , air convection requirements ($\dot{V}_E/\dot{V}_{\text{O}_2}$) doubled during exercise, indicating that compared with rest, pythons hyperventilate during exercise. The lizard *Varanus exanthematicus* (Wang et al., 1997) and several mammalian species (Wasserman et al., 1980; Pan et al., 1984; Longworth et al., 1989) have also been observed to hyperventilate during exercise. The exercise-induced increase in systemic output for pythons is the product of a 130% increase in heart rate and a 30% increase in calculated stroke volume. Apparently, during activity, oxygen and carbon dioxide transport in the cardiovascular system becomes much efficient: oxygen extraction ($\text{ml O}_2 \text{ml}^{-1} \dot{Q}_{\text{sys}}^*$) more than triples and carbon dioxide carriage ($\text{ml CO}_2 \text{ml}^{-1} \dot{Q}_{\text{sys}}^*$) increases sixfold (Table 1).

Cardiopulmonary support during digestion

After feeding, pythons responded with the characteristic postprandial increase in metabolic rate (specific dynamic action). For pythons, the magnitude of this metabolic response is closely correlated with relative meal size, and for the meal size of 25% of snake body mass, the peak \dot{V}_{O_2} measured in this study was equivalent to that observed in previous studies (Secor and Diamond, 1995, 1997). The greater than 400% increase in \dot{V}_E experienced during digestion is the product of a large increase in breathing frequency (by 360%) and a much smaller increase in tidal volume (by 40%). These ventilatory increments experienced during digestion were significantly smaller than those experienced during fasting exercise, even

though \dot{V}_{O_2} levels were equal. Thus, the resultant air convection requirement ($\dot{V}_E/\dot{V}_{\text{O}_2}$) at peak digestive \dot{V}_{O_2} was less than half of that experienced at rest. In contrast to hyperventilating during exercise, pythons hypoventilated during digestion. Because these snakes have ingested a relatively large meal, the postprandial hypoventilation may simply be the result of the distended stomach constraining lung expansion. However, this is not supported by the observation that \dot{V}_E can increase substantially during postprandial exercise. It is more likely that the postprandial hypoventilation represents a change in ventilatory control, potentially resulting from the elevation in plasma $[\text{HCO}_3^-]$ that occurs in pythons during digestion (Secor and Diamond, 1995).

Concurrent with their postprandial increase in gas exchange, pythons also experience a noted increase in cardiac output. \dot{Q}_{sys}^* , an approximation of cardiac output, rose by 350% during digestion, as a result of relatively equal increases in heart rate (by 140%) and stroke volume (by 100%). The significantly higher \dot{Q}_{sys}^* that pythons attained during digestion compared with during fasting exercise is largely attributable to the significantly greater V_s^* during digestion. This greater V_s^* can partially be explained by the postprandial increase in heart mass (as much as 50%) that pythons experience (Secor and Diamond, 1995). Oxygen and carbon dioxide transport efficiencies increased above resting levels during digestion, but were significantly less than those observed during activity (Table 1).

Cardiopulmonary support during postprandial exercise

Studies on the individual and paired demands of exercise and digestion have largely focused on humans. For humans, levels of moderate exercise while fasted induce an increase in \dot{V}_{O_2} of 300–350%, whereas digestion alone causes a more modest 30% increase in \dot{V}_{O_2} (Segal and Gutin, 1983; Welle, 1984). When

occurring together, these demands result in a 16–54% further increase in \dot{V}_{O_2} over exercise-only values. It is uncertain for humans whether the response to digestion is simply additive to that of exercise. Metabolic responses to both activities have been found to be less than (Welle, 1984), equal to (Belko et al., 1986) or greater than (Segal and Gutin, 1983) that predicted on the basis of summing the individual responses of exercise and digestion. For exercising and digesting pythons, we asked (i) whether the response of one metabolic demand was dominant over that of the other, and (ii) whether the responses were additive.

During postprandial exercise (at 72 h post-feeding), \dot{V}_{O_2} and \dot{V}_{CO_2} rose to levels significantly greater than those during either exercise or digestion alone (Tables 1, 2). With activity, \dot{V}_{O_2} and \dot{V}_{CO_2} of digesting pythons had risen by 95% and 280%, respectively (Table 2). If the response to exercise and digestion were additive (i.e. the sum of the two independent responses), then \dot{V}_{O_2} and \dot{V}_{CO_2} of exercising pythons at 72 h post-feeding would be predicted to increase to 11.3 and 13.5 ml kg⁻¹ min⁻¹, respectively (Table 2, calculation of predicted values is explained in the footnote). The observed \dot{V}_{O_2} was 80% of the predicted value, whereas the observed \dot{V}_{CO_2} was 105% of that predicted (Table 2). Apparently, the response in \dot{V}_{O_2} is nearly additive, whereas the response in \dot{V}_{CO_2} is fully additive of the separate responses to exercise and digestion. For pythons exercising and digesting, the larger increase in \dot{V}_{CO_2} relative to the increase in \dot{V}_{O_2} resulted

in a significantly greater RER than observed during either exercise or digestion alone (Table 1).

The breathing frequency of pythons during digesting exercise was significantly greater than that during either fasting exercise or resting at 72 h post-feeding (Tables 1, 2), and did not differ from that predicted for an additive effect of these two metabolic demands (Tables 1, 2). In contrast, tidal volume during exercise and digestion was significantly less than that during exercise alone. There was, therefore, was no additive relationship between exercise and digestion for tidal volume (Tables 1, 2). Minute ventilation during exercising at 72 h post-feeding, statistically equivalent to that during exercise alone, did not differ from the predicted value assuming an additive relationship (Table 2). Ventilatory convection requirements suggest that the postprandial exercise response is dominated by exercise rather than digestion: \dot{V}_E/\dot{V}_{O_2} and \dot{V}_E/\dot{V}_{CO_2} both increase significantly (both $P<0.03$) with exercise above 72 h post-feeding values, and the magnitude of \dot{V}_E/\dot{V}_{O_2} , in comparison with that of resting fasted animals, is suggestive of hyperventilation (Table 1).

During postprandial exercise, heart rates increased by 17% and 20% (both $P<0.002$), respectively, over fasting exercise and resting 72 h post-feeding rates, but these increases were not enough to demonstrate an additive response (Tables 1, 2). Pythons did not significantly increase their \dot{Q}_{sys}^* with exercise at 72 h post-feeding, indicating that cardiac output

Table 2. Two sets of paired comparisons of ventilatory and cardiovascular variables from juvenile Burmese pythons (Python molurus) at 72 h post-feeding

Variable	N	Resting at 72 h post-feeding	Exercising at 72 h post-feeding	Paired <i>t</i> -test: resting at 72 h post-feeding versus exercising at 72 h post-feeding <i>P</i>	Predicted exercising at 72 h post-feeding	Paired <i>t</i> -test: actual versus predicted exercising at 72 h post-feeding <i>P</i>
\dot{V}_{O_2} (ml kg ⁻¹ min ⁻¹)	5	4.5±0.1	9.0±0.4	<0.0001	11.3±0.4	0.045
\dot{V}_{CO_2} (ml kg ⁻¹ min ⁻¹)	5	3.8±0.2	14.2±1.4	0.002	13.5±1.1	0.76
Breathing frequency (breaths min ⁻¹)	5	6.0±0.8	18.1±1.0	<0.0001	18.9±1.1	0.46
Tidal volume (ml kg ⁻¹)	5	20.2±1.6	33.7±2.8	0.003	47.8±2.9	0.026
\dot{V}_E (ml kg ⁻¹ min ⁻¹)	5	118±9	604±41	0.0002	750±56	0.14
Heart rate (beats min ⁻¹)	5	55.8±1.5	66.6±2.1	0.0016	87.4±1.0	0.0011
Left systemic blood flow (ml kg ⁻¹ min ⁻¹)	5	34.4±2.0	37.3±0.8	0.21	55.1±1.7	0.0001
Right systemic blood flow (ml kg ⁻¹ min ⁻¹)	4	31.7±4.2	24.0±1.5	0.22	45.7±4.5	0.025
\dot{Q}_{sys}^* (ml kg ⁻¹ min ⁻¹)	4	58.9±2.2	61.9±3.8	0.65	95.7±2.6	0.006
V_s^* (ml kg ⁻¹)	4	1.18±0.08	0.95±0.038	0.084	1.38±0.06	0.008
O ₂ extraction (ml O ₂ ml ⁻¹ \dot{Q}_{sys}^*)	4	0.07±0.01	0.15±0.01	0.003	0.17±0.01	0.40
CO ₂ carriage (ml CO ₂ ml ⁻¹ \dot{Q}_{sys}^*)	4	0.06±0.01	0.22±0.03	0.005	0.21±0.02	0.74

The first set compares measurements made of snakes resting at 72 h post-feeding with exercising at 72 h post-feeding.

The second set compares the actual measured values during exercise at 72 h post-feeding with the predicted values based upon an additive relationship of responses to exercising fasted and digesting at 72 h post-feeding.

Predicted values for exercising at 72 h post-feeding for each snakes were calculated as: (exercise while fasted) plus (resting at 72 h post-feeding minus resting while fasted) (see Table 1).

Values are presented as means ± 1 S.E.M.; *N* refers to the number of individual snakes.

Results of paired *t*-tests are presented as *P* values.

\dot{V}_E , minute ventilation volume; \dot{Q}_{sys}^* , cardiac output; V_s^* , stroke volume.

had already reached a maximum during digestion alone (Table 2). Stroke volume of digesting snakes did not change significantly with exercise, and was the same as that experienced during exercise alone (Table 1). Gas transport efficiencies increased significantly (all $P < 0.005$) when digesting pythons exercised: oxygen extraction doubled and carbon dioxide carriage more than tripled from the resting 72 h post-feeding values (Table 2).

Cardiopulmonary limits to metabolism

Studies on mammals, birds and fishes have usually shown that $\dot{V}_{O_{2max}}$ is attained during locomotion (Brett, 1972; Seeherman et al., 1981; Suarez, 1992). In contrast, some frogs and snakes have demonstrated higher \dot{V}_{O_2} and higher endurance at elevated \dot{V}_{O_2} during non-locomotor activities. Male treefrogs *Hyla versicolor* achieve higher \dot{V}_{O_2} during calling than during locomotion (Taigen and Wells, 1985). Whereas in the present study the \dot{V}_{O_2} of pythons during digestion of meals equal to 25% of body mass did not differ from locomotor $\dot{V}_{O_{2max}}$, \dot{V}_{O_2} during the digestion of larger meals ($\geq 35\%$ of body mass) can exceed locomotor $\dot{V}_{O_{2max}}$ by as much as 200% (Secor and Diamond, 1997). In addition, pythons are able to maintain locomotor $\dot{V}_{O_{2max}}$ for only approximately 10 min, whereas during digestion they can sustain a minimum \dot{V}_{O_2} of $7.6 \text{ ml kg}^{-1} \text{ min}^{-1}$ (locomotor $\dot{V}_{O_{2max}}$ of this study) for three consecutive days (Secor and Diamond, 1997).

Assuming that maximal rates of aerobic metabolism ($\dot{V}_{O_{2max}}$) are attained only during strenuous locomotor activities, the design of the pulmonary, cardiovascular and/or skeletal muscular system has been scrutinized for the limiting step (or steps) of aerobic metabolism (Taylor et al., 1981; Suarez, 1996; Wagner, 1996). The recent findings for pythons and previously for frogs indicate that maximal levels of gas transport and cardiopulmonary function (including breathing rate, heart rate, V_{s^*} and \dot{Q}_{sys^*}) may not be attained during locomotor activity. Therefore, cardiopulmonary factors cannot be asserted to limit (or co-limit) oxygen consumption or gas exchange during locomotor activity in these animals. For pythons, locomotor $\dot{V}_{O_{2max}}$ is apparently not set by cardiopulmonary limits but is possibly dictated by the oxygen diffusion and/or aerobic capacity of the skeletal muscle. Likewise, during digestion, pulmonary ventilation cannot be asserted to be a limiting factor, since postprandial exercise results in a rapid and marked increase in both breathing frequency and tidal volume. When postprandial animals begin exercising, cardiac output does not increase, but the efficiency of cardiovascular gas transport does increase. Therefore, either cardiac capacity and/or peripheral oxygen diffusion or utilization in the gut may limit digestive $\dot{V}_{O_{2max}}$. Finally, as is now clear, locomotor activity does not elicit $\dot{V}_{O_{2max}}$ in these pythons or in certain species of anurans. Therefore, the hypothesis of symmorphosis (Taylor et al., 1981) cannot be adequately examined or tested in ectothermic vertebrates in general by observations during locomotor activity alone.

This study was financially supported by a NSF Grant (IBN-9727762) to J.W.H. and A.F.B. and by a NIH grant (GM-14772) to Dr Jared Diamond. We are grateful to Dr Colleen

Farmer for technical assistance in this project and to Dr Jared Diamond for his helpful comments.

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