ORIGINAL RESEARCH

Hyperoxia reduces costs of digestion in snakes: potential bioenergetic consequences of the paleoatmosphere

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Abstract: Oxygen levels in the Earth's atmosphere fluctuated dramatically during the Phanerozoic eon, and may have reached concentrations that were 60% higher than current levels. These atmospheric changes have engendered much speculation about the relationship between O_2 availability and major evolutionary events among animals (eg, insect gigantism, flapping flight, endothermy). With the exception of studies of exercising humans, a limited number of investigations have examined the potential effects of hyperoxic conditions on physiologic performance variables in animals. Given that the energetic costs of maintenance and digestion can account for the majority of the total energy budget of snakes, this study was conducted to determine the effect of simulated paleoatmospheric oxygen concentration on the metabolic rates of postabsorptive and postprandial snakes. A series of three repeated-measures trials conducted on Western diamondback rattlesnakes under oxygen concentrations ranging from 21% to 50% revealed that hyperoxia did not affect resting metabolic rates, but that 35% O_2 was sufficient to reduce specific dynamic action by an average of 11%. Significant increases in respiratory exchange ratios were also observed in postprandial snakes. Given the degree to which extant snakes are energy-limited, such an energetic benefit may have had significant implications for the feeding behaviors and life histories of ancient snakes as they radiated from their lizard ancestors.

Keywords: oxygen limitation, pitvipers, postprandial calorigenesis, rattlesnakes, respiratory quotient, specific dynamic action

Introduction

All terrestrial animals rely on atmospheric oxygen to carry out the physiologic functions necessary for life. The presumption that the oxygen concentration of the Earth's atmosphere has remained constant over the Phanerozoic eon, espoused by the Gaia hypothesis,[1–3] has come under increased scrutiny by recent geochemical models consistently reporting that the oxygen concentration on Earth has fluctuated dramatically over the past 500 million years.[4–9] Although the present atmosphere contains approximately 21% oxygen, ancient atmospheric oxygen concentrations are likely to have exceeded 30% around 300 million years before the present, and again as recently as 100 million years before the present[7,8] (Figure 1).

It has been repeatedly suggested that paleoatmospheric O_2 pulses and geological periods of elevated oxygen concentration have been responsible for shaping the morphology and physiology of metazoans through evolutionary time.[10,11] Specifically, O_2 pulses are thought to have permitted the gigantism of insects,[12,13] as well as the coincidental development of endothermy[14,15] and flapping flight among birds and

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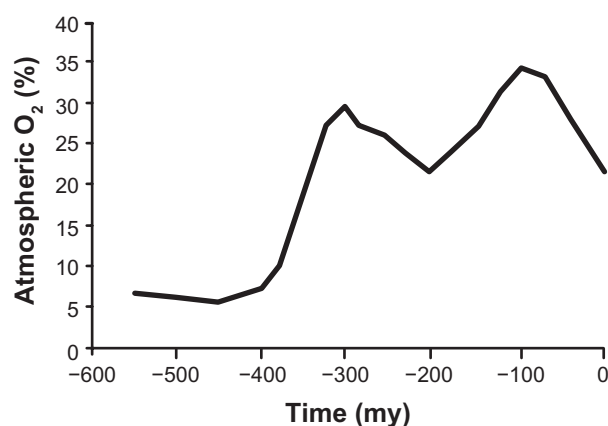
Figure 1 Paleoatmospheric O_2 levels over the past 500 million years. Drawing adapted from geological models.[8] Two large O_2 pulses peaking at approximately 300 and 100 million years before the present.

mammals.[16,17] Often overshadowed by discussions about the radiation of birds and mammals is the coincidental adaptive radiation of snakes from their lizard ancestor.[18,19] In fact, since the first snakes appeared over 80 million years before the present, they have come to account for nearly half of all extant reptile species and can be found on six continents.[20–24] Is it possible that, like insects, birds, and mammals, the evolutionary radiation of snakes may have been facilitated by paleoatmospheric conditions? This study examines the impact that hyperoxia has on one of the most energetically demanding activities of snakes, namely specific dynamic action (SDA).

The energy assimilated from an animal's diet can be routed to several potential sinks, including costs of digestion (ie, SDA), maintenance (resting metabolic rate), activity/locomotion, growth, storage, and reproduction.[25,26] The proportions of energy allocated to each of these sinks are dictated by each species' life history, and are shaped by natural selection over evolutionary time.[27] The energetic costs of SDA have been quantified in hundreds of invertebrates and vertebrates, and have been shown to vary widely among species.[28,29] Over the past decade, snakes have become a model system for investigating digestive energetics because of their comparatively large and prolonged SDA responses,[30] and several energetic models of pitvipers have determined that the combined costs of resting metabolic rate and SDA are comparatively high, and accounting for the majority of their total assimilated energy.[31–35]

During elevated metabolic states, an animal's physiologic performance is believed to be limited by one of several possible diffusive and convective steps as molecular oxygen enters the body through the lungs (or gills) and is ultimately reduced at the level of the mitochondria.[36–39] Because the diffusion of molecular oxygen across respiratory surfaces is concentration-dependent,[40] several physiologists have speculated that pulmonary diffusion could be a limiting step regulating levels of aerobic metabolism, particularly during elevated metabolic states.[41–45]

Numerous studies have shown that aerobic performance can be affected by hyperoxia (Table 1), and two recent studies of fish have shown that the total costs and duration of SDA are increased during exposure to hypoxia.[46,47] Is it possible that a release from oxygen limitation could influence the costs of SDA? This question is addressed by studying the digestive energetics of the Western diamondback rattlesnake (*Crotalus atrox*), a widely distributed, heavy-bodied pitviper. The predictions that hyperoxia will reduce resting metabolic rates in postabsorptive snakes, respiratory exchange ratios of snakes, and energy required to digest a given meal, are examined.

The respiratory exchange ratio refers to the ratio of an animal's rate of CO_2 production to O_2 consumption.[48] Because the oxidation of different physiologic fuels (eg, carbohydrates, proteins, and fats) yields different respiratory exchange ratios, these metrics are often used qualitatively to track changes in fuel utilization. While fasting animals generally decrease their respiratory exchange ratio as they progressively increase rates of lipid oxidation,[49–53] much less is known about how the respiratory exchange ratio is affected by digestion.[40,54–58]

Vipers and pitvipers have been used as models for studying resource limitation for three major reasons. First, their resting metabolic rates are comparatively low, suggesting that these animals may have evolved in conditions where chemical energy is limiting.[59–65] Second, their SDA responses tend to be larger than in most other snakes.[28,66–69] Third, resting metabolic rates and postprandial energetic responses are well characterized in these animals under present atmospheric conditions.[34,70–72]

This study was conducted to examine the bioenergetic consequences of living in a hyperoxic atmosphere. Because it is impossible to make physiologic measurements on extinct species in their contemporary environments, we examine how an extant species reacts to artificial atmospheric conditions that mimic ancient atmospheres.

Methods
Animals
Adult and subadult *Crotalus atrox* (n = 20) collected from various localities throughout their range (body

Table 1 Studies reporting performance variables in animals under varying hyperoxic conditions

Organism	O_2 condition	Exposure	Result	Reference
Bats	35%	acute*	no improved hovering performance in hypodense air (H_2)	120
Cat mesentery	hyperoxia	acute	no changes in blood flow in an in vitro preparation	121
Chicken eggs	60%	chronic	increased development rate and larger embryos	122
Drosophila adults	40%	chronic	reduced life span from approximately 55 to 35 days	115
Drosophila pupae	40%	chronic	caused shorter/fatter adult bodies	123
Frog eggs	99% (7 ATM)	acute	incomplete or retarded gastrulation	124
Guinea pigs	45%	acute	accelerated clearance of bacterial infection (via increased neutrophil activity)	125
Humans	60%	acute	no changes in ventilation, heart rate, or lactate during exercise	14
Humans	50%	chronic	no toxic effects observed	111
Humans	100%	acute	vasoconstriction but increased peripheral pO_2	126
Humans	hyperoxia	acute	downregulation of vascular endothelial growth factor	127
Humans	50%	acute	increased O_2 debt recovery	79
Humans	100%	acute	increased O_2 delivery and ultimately VO_2 max of leg	42
Humans	21% (1.4 ATM)	acute	reduced O_2 deficit and lactate accumulation at submaximal exercise, and increased performance at VO_2 max	128
Humans	70%	acute	increased ventilatory threshold at near maximal exercise	45
Humans	100%	acute	exercising humans did not influence VO_2, O_2 delivery, or blood pressure of leg, but reduced blood flow	129
Humans (lung disease)	60%	acute	reduced heart rate and ventilation requirements during exercise	130
Hummingbirds	33%	acute	females had slight improvement in hovering performance in hypodense gas, O_2 diffusion was not limited	131
Lambs	33%–46%	acute	increased summit metabolism (ie, cold-induced VO_2 max), improved cardiac output	132
Lizard eggs	30%	chronic	no difference in egg incubation times	133
Mouse pups	85%	chronic	retarded histologic development in lungs, and reduced lung compliance	134
Quail eggs	60%	acute	no changes in embryonic wet mass or rates of O_2 consumption	135
Rat pups	95%	acute	reduced weight gain and increase oxidase enzyme levels in lungs	110
Rat pups	60%	chronic	caused reduced hypoxia tolerance	136
Rat pups	60%	chronic	adults had attenuated physiological responses to hypoxia	137
Rats	85%	acute	reduced capillary vascularization and hematocrit	138
Rats	100%	chronic	partial hemolysis of red blood cells, altered hemoglobin levels, edema of alveolar tissues	139
Rats	100%	acute	reduced cardiac output, cerebral blood flow, and heart rates	140
Rats	42%	acute	no abnormal histology	141
Rats (wounded)	100% (2.5 ATM)	acute	increased vascular endothelial growth factor and reduced lactate	112
Sea snake	100%	acute	reduced ventilation rates	142
Tortoise/turtle	100%	acute	reduced ventilation rates	143
Turtles	100%	acute	reduced ventilation rates	92

Note: *Acute exposure refers to less than 14 days.

masses 339–1145 g) were donated to this project by E Taylor (Arizona State University, AZ), J Bibby (Fort Worth, TX), and C Guyer (Auburn University, AL). Prior to the experimental feeding trials, snakes were housed individually in locking plastic cages, and acclimated to laboratory conditions that simulated their active/feeding season (27 ± 1°C; 12 hours light,12 hours dark) for a minimum of 12 weeks. During the acclimation period, all snakes consumed at least eight meals consisting of commercially raised prekilled feeder mice (Rodent Pro, Inglefield, IN), and were fasted for two weeks prior to feeding experiments to ensure that they were postabsorptive.

Experimental design

This study consisted of a series of three experiments, each employing a complete, repeated-measures design, thereby facilitating comparisons of metabolic responses under different oxygen concentrations.

In experiment 1, animals (n = 8; 830 g, standard deviation [SD] ± 187 g) were fed two prekilled mice (combined mass of approximately 47 g; approximately 6% relative prey mass). Immediately after ingesting the meals, each snake was placed into a 4 L polypropylene respirometry chamber and exposed to one of three oxygen concentrations (ie, 21%, 35%, and 50%) wherein

postprandial metabolic rates were quantified each hour during the subsequent 120 hours.

Several variables, including body mass, meal mass, resting metabolic rate (RMR), energy of SDA, time to peak SDA (T_{peak}), scope of postprandial metabolic increase (SDA_{scope}), SDA coefficient (SDA_{coeff}), and (RER_{peak}) were compared among the three treatment levels using repeated-measures one-way analysis of variance (ANOVA), and Fisher's protected least significant difference. The SDA, SDA_{coeff}, and SDA_{scope} were calculated using the following standard equations:[28,29]

$$SDA = (ml_{0_2\,consumed} * K) - (RMR * t)$$

$$SDA_{scope} = SDA_{peak}/RMR$$

$$SDA_{coeff} = \frac{SDA}{E_{ingested}}$$

where K is 20.28 kJ*ml O_2^{-1},[31,51,73–75] t is the duration of the trials in hours, SDA_{peak} is the highest observed postprandial metabolic rate, and $E_{ingested}$ is 7.02 kJ*g^{-1}.[76] Resting metabolic rate was calculated as the mean metabolic rate of postabsorptive animals at rest, measured hourly for 24 hours prior to each feeding trial at the same oxygen concentration as each of the feeding treatments. The first hour of resting metabolic rate measurements were omitted from the analyses to avoid the inclusion of short-term stress responses associated with handling.

Experiment 2 employed the eight animals from the first experiment (n = 8; 867 ± 186 g), but used larger meal sizes (ie, three mice totaling approximately 101 g; approximately 12% relative prey mass). After a three-week period of fasting, the snakes were fed a meal and exposed to 35% O_2 for 144 hours. Three weeks later, the snakes were fed a similar-sized meal and subjected to 21% O_2 over the subsequent 144 hours.

Experiment 3 employed a second set of smaller snakes (n = 12; 410 ± 76 g). The snakes were fasted for three weeks and fed meals consisting of three mice with combined masses of approximately 64 g (approximately 15% relative prey mass). Snakes were subjected to 35% O_2, and SDA measurements were also made over the subsequent 144 hours. Three weeks later, the feedings and SDA measurements were repeated at 21% O_2. The same variables were monitored as in the trial 1, but one-tailed, paired t-tests were used to compare responses between 21% and 35% O_2. Because of the unexpectedly high variability in responses among snakes subjected to 50% O_2 during trial 1, the 50% O_2 treatment was not used in experiments 2 and 3. The total duration of the

SDA response was not analyzed because of the difficulty in reliably distinguishing SDA from small diel variations in resting metabolic rate.[77]

These experiments were conducted in accordance with University of Arkansas Animal Care and Use Committee protocol number 080107.

Gas control and measurement

A stream of dry O_2 generated using a Devilbiss-515® O_2 concentrator (Devilbiss, Somerset, PA) capable of producing a constant supply of 95% O_2 at up to 5 L per minute was combined with room air inside a mixing chamber ported with one-way valves (Figure 2). The gas inside the mixing chamber was circulated using a standard 90 mm brushless computer fan and was pumped through a Drierite™ column (Cole-Parmer, Vernon Hills, IL). The dried gas mixture then entered a second surge tank to dampen the oscillations caused by the diaphragm pump and to minimize potential fluctuations in O_2 concentration.

The gas mixture then entered an MF-8 manifold (Sable Systems™, Las Vegas, NV) where it was split into eight gas streams that passed through the individual metabolic chambers. The first metabolic chamber was always left unoccupied and served as a measure of baseline (ie, incurrent) gas concentrations. Gases exiting the metabolic chambers were pushed through a Sable Systems RM-8 multiplexer programmed to divert individual gas streams into a subsampling junction serially every 7–8 minutes, thereby allowing the metabolic rate of each animal to be measured hourly.

A subsample of each gas stream was pulled through a second desiccant column (approximately 60 mL/min) followed by an Ametek™ (Paoli, PA) CD-3A carbon dioxide analyzer (spanned with dry N_2 and 10% CO_2; Air Liquid America Specialty Gases, Pasadena, TX) and an Ametek™ S-3A O_2 analyzer (spanned using N_2 and dry air) using a vacuum pump and flow controller. Oxygen and carbon dioxide concentrations were recorded constantly.

All measurements of O_2 consumption and CO_2 production were calculated using modified Fick's equations corrected for STP,[60,70,72,78] assuming that rates of gaseous nitrogen flux during hyperoxic treatments were not significantly different from those under normoxia; see calculations and discussions in previous studies.[79–81] In order to standardize for differences in SDA responses resulting from the slight variations in meal sizes among animals within respective experiments, the measured SDA responses were corrected to the mean meal mass for each experiment assuming an isometric SDA response over a relatively narrow range of meal sizes differing by less than 4 g.
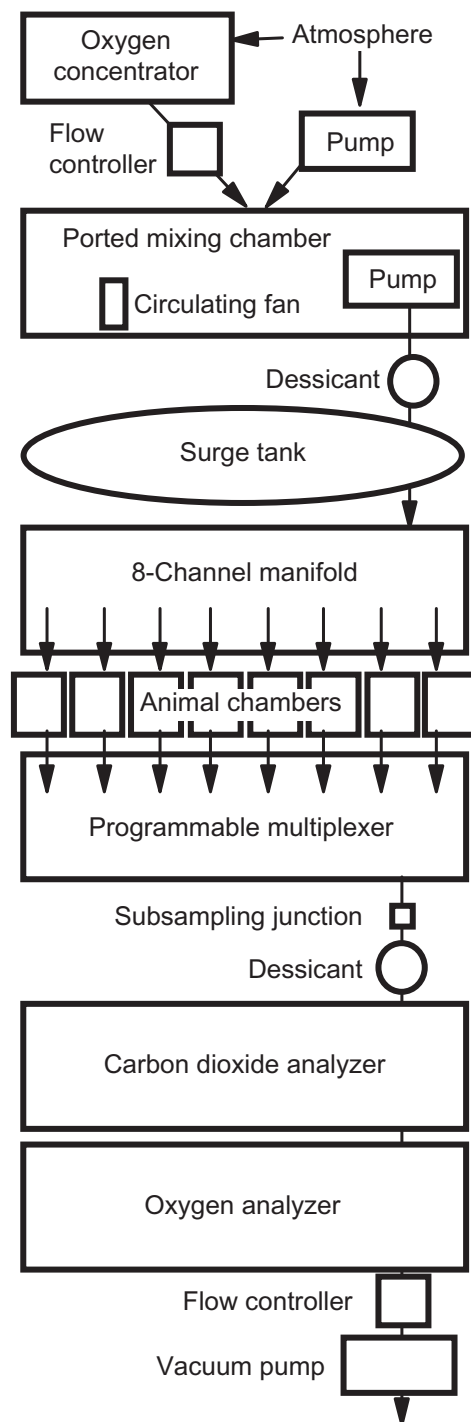
Figure 2 Hyperoxia-generating respirometry system. Open-flow respirometry system capable of producing controlled hyperoxic gas mixtures (ranging from 21% to 70% O_2) to eight metabolic chambers at flows up to 2 L/min.

A pilot study designed to monitor the consistency of the oxygen concentration gas streams and flow rates through animal chambers found less than a 0.01% change in oxygen concentration over a one-hour period at 35% O_2 and a 0.02% fluctuation at 50% O_2. Drift in oxygen concentration was always less than 0.001% over a one-hour period. Flow rates

through empty metabolic chambers ranged between 100 and 400 mL/min and were continually measured using a Sable Systems FoxBox. During a 24-hour period flow rates did not fluctuate by more than 1% of their initial value.

Results
Experiment 1
Repeated-measures ANOVAs comparing resting metabolic rate and SDA results revealed that only T_{peak} differed among the three treatments ($P = 0.010$); no differences in resting metabolic rate were detected. Post hoc tests showed that T_{peak} responses at 35% O_2 were significantly different from those at 21% and 50% O_2. Two-tailed pairwise comparisons of responses measured at 21% and 35% O_2 were statistically significant for SDA, SDA_{corr}, T_{peak}, SDA_{scope}, and SDA_{coeff} (Table 2, Figure 3A).

Experiment 2
One-tailed, pairwise comparisons revealed that SDA, SDA_{corr}, T_{peak}, SDA_{scope}, and SDA_{coeff} were significantly reduced in 35% O_2 (Table 2, Figure 3B); no differences in resting metabolic rate were detected. Respiratory exchange ratios reduced sharply upon ingestion and then increased rapidly during the first 10 hours of digestion. A less rapid increase in respiratory exchange ratio toward postabsorptive values was observed during the final stages of SDA (Figure 4A).

Experiment 3
One-tailed, pairwise comparisons showed that SDA, SDA_{corr}, SDA_{scope}, and SDA_{coeff} were significantly reduced under 35% O_2 (Table 2, Figure 3C), but no differences in resting metabolic rate were observed between normoxic and hyperoxic treatments. As in the second experiment, respiratory exchange ratios increased sharply during the first 10 hours of digestion from values of approximately 0.50 to 0.60, before ultimately increasing at a more gradual rate reaching values of 0.70–0.80 near the end of the SDA response (Figure 4B).

Discussion
The results of this experiment did not support the prediction that resting metabolic rate was influenced by gas mixtures containing up to 50% O_2, but do show that postprandial energy expenditure was influenced by inspired oxygen concentration. In all experiments, a level of 35% O_2 was sufficient to reduce three interrelated variables associated with postprandial energetics, ie, total energy required for meal digestion and absorption (ie, SDA), peak postprandial metabolic rate (ie, SDA_{scope}), and the relationship between
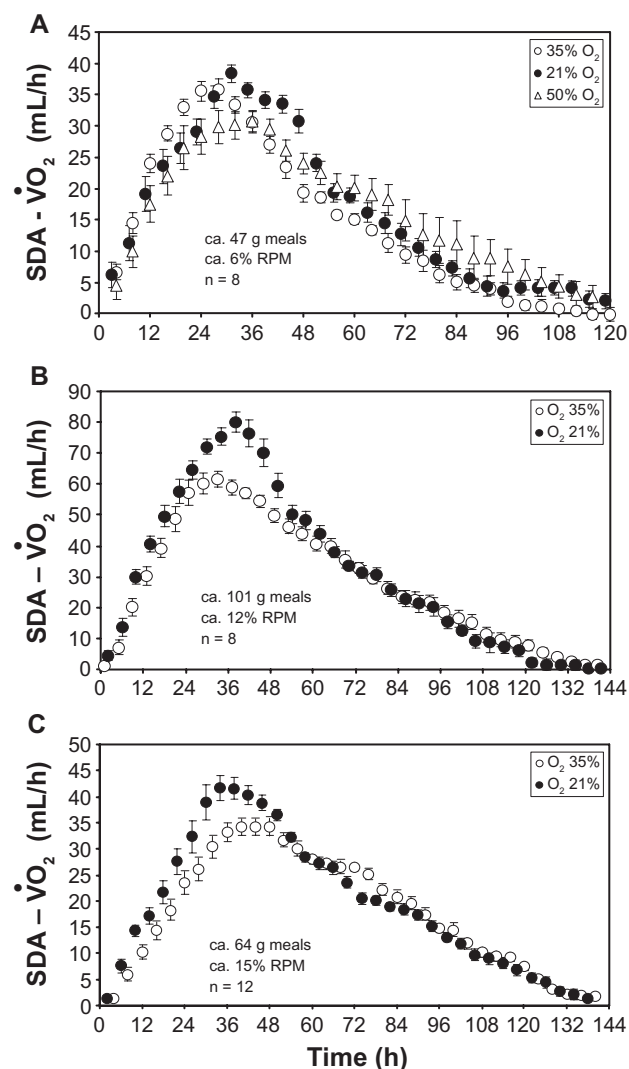
Table 2 Results of feeding experiments during which pitvipers were allowed to digest meals under normoxia and hyperoxia

	21%	35%	50%	P
Experiment 1				
Mass (g)	811 [188]	836 [186]	843 [187]	
RMR (mL O₂/hour)	23.9 [6.8]	22.7 [6.67]	24.7 [6.4]	
Meal (g)	46.9 [2.2]	48.2 [2.0]	46.9 [1.8]	
SDA (kJ)	52.9 [7.0]	46.2 [2.6]	52.3 [12.6]	0.035*
SDA_{corr} (kJ)	56.5 [8.3]	48.0 [2.9]	56.1 [16.2]	0.015*
T_{peak} (hours)	33.0 [5.4]b	24.1 [1.3]a	32.6 [8.5]b	0.003*
SDA_{scope}	3.6 [0.8]	3.5 [1.1]	3.3 [0.9]	0.021*
SDA_{coeff} (%)	16.1 [2.2]	13.9 [0.8]	15.9 [4.6]	0.015*
RER_{peak}	0.668 [0.029]	0.665 [0.057]	0.671 [0.035]	
	n = 8	n = 8	n = 8	
Experiment 2				
Mass (g)	879 [183]	856 [188]	–	
RMR (mL O₂/hour)	27.5 [3.5]	28.5 [5.7]	–	
Meal (g)	100.9 [4.2]	101.9 [4.8]	–	
SDA (kJ)	118.0 [11.4]	102.6 [15.1]	–	0.029
SDA_{corr} (kJ)	117.0 [10.5]	100.8 [15.1]	–	0.030
T_{peak} (hours)	37.1 [3.3]	32.3 [4.7]	–	0.014
SDA_{scope}	5.1 [1.0]	4.2 [0.8]	–	<0.001
SDA_{coeff} (%)	16.7 [1.5]	15.3 [2.1]	–	0.003
RER_{peak}	0.642 [0.014]	0.649 [0.013]	–	
	n = 8	n = 8		
Experiment 3				
Mass (g)	412 [77]	408 [74]	–	
RMR (mL O₂/hour)	14.9 [2.1]	15.6 [2.5]	–	
Meal (g)	64.0 [1.6]	63.4 [1.7]	–	
SDA (kJ)	73.9 [9.4]	69.4 [7.6]	–	0.016
SDA_{corr} (kJ)	73.9 [5.4]	69.3 [7.7]	–	0.019
T_{peak} (hours)	39.3 [5.2]	39.5 [4.2]	–	
SDA_{scope}	5.1 [1.0]	4.2 [0.8]	–	0.002
SDA_{coeff} (%)	16.6 [1.4]	15.4 [1.7]	–	0.006
RER_{peak}	0.658 [0.034]	0.675 [0.025]	–	
	n = 12	n = 12		

Notes: Values in brackets refer to one standard deviation. Relevant assumptions are explained in the Methods section. Letters beside values refer to responses that were statistically different according to post hoc tests. Only significant P values are shown. *Two-tailed, paired t-tests between 21% and 35% O₂ were used; all other P values refer to one-tailed, paired t-tests.
Abbreviations: RMR, respiratory metabolic rate; SDA, specific dynamic action; T_{peak}, time to peak SDA; SDA_{scope}, scope of postprandial metabolic increase; SDA_{coeff}, SDA coefficient; RER_{peak}, respiratory exchange ratio at peak SDA; SDA_{corr}

Figure 3 Specific dynamic action responses under normoxia and hyperoxia in pitvipers measured during the three experiments. **A**) Snakes (n = 8) ingesting approximately. 47 g mouse meals subjected to three O₂ treatment levels. **B**) Snakes (n = 8) ingesting approximately 101 g mouse meals subjected to two O₂ treatment levels. **C**) Snakes (n = 12) ingesting approximately 64 g mouse meals subjected to two O₂ treatment levels. Error bars refer to ± one standard error of the mean. For clarity, data points are slightly offset along the x-axis.

ingested energy and energy required for digestion and assimilation (ie, SDA_{coeff}). On average, the animals required 11% less energy to process meals under 35% O₂ compared with normoxia. For pitvipers, that might only consume eight or nine meals during an entire feeding season,[32] the "savings" realized from digestion under hyperoxic conditions could translate to an annual "free" meal. Two of the three experiments showed that hyperoxic conditions resulted in lower T_{peak} values, supporting the prediction that hyperoxia influences the time course of SDA; however, because the difference was not significant in the final experiment, it remains possible that this response could be a statistical artifact.

The specific mechanisms underlying the reduction in SDA under hyperoxia remain untested, but might involve a reduction in lactate production[58,82–85] and the subsequent costs of gluconeogenesis,[28,86–89] as well as reduced ventilatory[40,90–93] and circulatory costs.[94–98] Future experiments designed to monitor the effects of hyperoxia on blood oxygen concentration, ventilation, heart rates, and lactate flux will be needed to examine each of these possibilities.
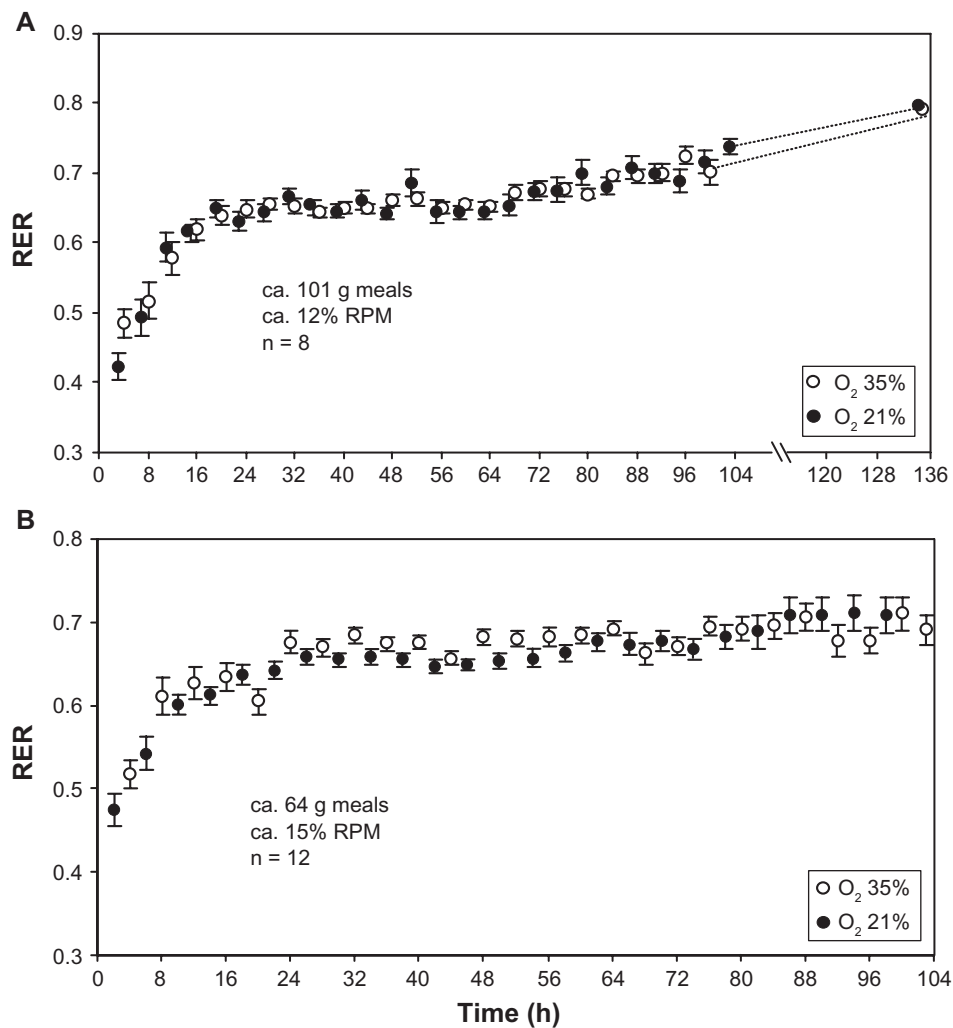
Figure 4 Respiratory exchange ratios in postprandial snakes subjected to normoxia and hyperoxia. Measurements of respiratory exchange ratio made in postprandial pitvipers subjected to two O_2 treatment levels. **A**) Snakes n = 8 ingesting approximately 101 g mouse meals. **B**) Snakes (n = 12) ingesting approximately 64 g meals. Error bars refer to ± one standard error of the mean. For clarity, data points are slightly offset along the x-axis.

Given that the meals used in these experiments were not near the upper limit that these animals can ingest, it may also be worthwhile to examine how hyperoxia influences snakes digesting extremely large meals (eg, relative prey masses in the range 50%–100%) where SDA$_{scope}$ may exceed a value of 20.[29,30,99,100]

No differences between respiratory exchange ratios during exposure to normoxia and hyperoxia were noted among postprandial or postabsorptive snakes. This finding did not support the prediction that hyperoxic conditions might preferentially favor the β-oxidation of endogenous fatty acids, thereby lowering respiratory exchange ratios among postabsorptive individuals, as has been documented in humans.[44,81] Previous studies of other snakes have documented general increases in respiratory exchange ratios during SDA[56,57] particularly in the early phases of digestion, there are no examples that repeatedly show such systematic

increases in the respiratory exchange ratio during digestion in the literature.

Three nonmutually exclusive mechanisms are proposed that might explain the comparatively low respiratory exchange ratios documented during the early phases of digestion. First, significant hypoventilatory responses have been reported in digesting, but not exercising, pythons.[54] Therefore, it is possible that the low respiratory exchange ratios during the early phases of digestion could stem from a respiratory acidosis caused by alkaline tides in the blood as hydrogen ions are routed into the gastric lumen.[55,99,101–104] A second possibility is that the snakes lost carbon via nonventilatory means, such as bicarbonate excretion.[51] It should be noted that although excretion events did not occur until the terminus of SDA, these snakes may have sequestered carbon-rich waste products (eg, bicarbonate and uric acid) in the distal gut.[105] A third possibility is that respiratory exchange

ratios was depressed as a result of an O_2 debt incurred during the ingestion process.[85,106–109]

Although no paleoatmospheric model has suggested that the Earth's atmosphere ever reached 50% O_2, it could be predicted that excessive O_2 enrichment might additively contribute to the responses observed in 35% O_2 treatments, but this was apparently not the case. In fact, the lack of similarity between responses measured at 50% and 35% O_2 suggests the likelihood that additional physiologic costs may have been incurred. If the partial pressures of O_2 in tissues increased beyond some limit, the snakes may have had to devote increasing energy to activities of antioxidants, including oxidases and catalases.[110–113] Several studies report the toxic effects of hyperoxygenation in animals are not realized until inspired O_2 levels exceed 50%,[111,113,114] but toxic effects have been reported in *Drosophila* chronically exposed to 40% O_2.[115]

It is possible that, like other animals, improvements in other performance variables (eg, locomotion) might be realized by snakes under hyperoxic conditions, and future studies will be needed to explore the extent to which other performance variables are influenced by hyperoxia, as well as examine generality of the feeding responses to hyperoxia reported here, particularly in other species, such as pythons, that also devote a large proportion of their ingested energy to digestive costs.[29,30] It will also be useful to examine whether similar responses are observed in individuals reared under or subjected to chronic hyperoxia.

For animals that are considered to be low-energy specialists, like many snakes, any savings in maintenance and/or food processing costs could have substantial cumulative effects on survival and reproductive output, and ultimately their overall fitness.[31,32,116–119] An understanding of the relationships between the environment and the physiology of ancient animals is not only requisite for accurate paleo-ecologic reconstructions, but also necessary to understand concomitant evolutionary trends that may have been shaped by paleoatmospheric changes.[10–12]

Acknowledgment

Disclosure

The author reports no conflict of interest in this work.

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