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Loss of the Ability to Control Right-to-Left Shunt Does Not Influence the Metabolic Responses to Temperature Change or Long-Term Fasting in the South American Rattlesnake *Crotalus durissus*

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ABSTRACT

In the undivided ventricle of noncrocodilian reptiles, the blood perfusing the systemic circulation is a controlled combination of oxygenated pulmonary blood, flowing from left to right across the heart, and relatively deoxygenated systemic blood, flowing from right to left. A clear inverse correlation has been experimentally established between metabolic demand and the magnitude of right-to-left cardiac shunt in several reptile groups. Unilateral left vagotomy renders the single effective pulmonary artery of the South American rattlesnake (*Crotalus durissus*) unable to adjust the magnitude of blood flow to the lung. This provides a unique model for investigation of the long-term consequences of abolition of the cardiac shunt in a squamate reptile. Rattlesnakes—vagotomized on the left or right side or sham operated—were exposed to long-term food deprivation or temperature change. Loss of control of the cardiac shunt following selective vagotomy did not change the progressive decrease in body mass or the onset of identifiable fasting stages. Resting metabolic rate and the increase in oxygen uptake measured during spontaneous or forced activity were also unchanged. The responses to reductions in temperature (from 30° to 20° or 15°C) in adult snakes or juvenile snakes were similarly unaffected by vagal transection. These data support rejection of the hypothesis that adjustment of the cardiac shunt is central to the control metabolic rate in squamate reptiles.

Introduction

Many snakes are sit-and-wait predators, fasting for long and unpredictable periods (Greene 1983). Because fasting animals rely exclusively on energy stores, it may be advantageous to reduce resting metabolic rate (RMR) to extend their fasting tolerance (Wang et al. 2006; McCue 2007, 2008). It has been suggested repeatedly that reptiles may reduce RMR through the generation of low arterial oxygen levels because of their ability to shunt blood from the right to left (R-L) side of the heart. This causes oxygen-poor blood to bypass the lungs and re-enter the systemic circulation (e.g., Shelton and Burggren 1976; Burggren 1987; Hicks and Wang 2004). Thus, it is characteristic that resting undisturbed reptiles exhibit large R-L shunts, while the R-L shunt is reduced when metabolic rate increases during activity or with elevated body temperature (Shelton and Burggren 1976; Burggren and Shelton 1979; Butler et al. 1984; Burggren 1987; Hicks and Wang 1996, 2012; Wang et al. 1997). In anesthetized turtles (*Trachemis scripta*), experimental generation of an R-L shunt reduced oxygen consumption (Hicks and Wang 1999, 2004; Platzack and Hicks 2001). However, fully recovered *T. scripta* does not reduce metabolic rate when prevented from increasing pulmonary blood flow during lung ventilation (Wang and Hicks 2008).

The South American rattlesnake (*Crotalus durissus*) is endowed with a typical squamate reptilian cardiovascular system in which the undivided ventricle (Jensen et al. 2010) allows for large cardiac R-L shunts whenever pulmonary vascular resistance increases relative to systemic vascular resistance (Galli et al. 2007). *Crotalus durissus* spontaneously exhibits large R-L shunts when resting undisturbed, particularly at low body temperatures (Wang et al. 1998; Taylor et al. 2009; Leite et al. 2013). The typical parasympathetic regulation of pulmonary blood flow in this species, however, differs from other noncrocodilian reptiles. Rattlesnakes, in common with some other species of snakes, possess a single functional lung. The single pulmonary artery is innervated by the left branch of the vagus nerve that acts to cause vasoconstriction, thus regulating pulmonary blood

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flow and consequent cardiac shunt patterns (Taylor et al. 2009; Leite et al. 2013). Sectioning the left vagus nerve causes an irreversible increase in pulmonary blood flow and prevents the generation of large R-L shunts while leaving intact parasympathetic innervation of other visceral organs, including the heart via the right vagus nerve (Taylor et al. 2009; Leite et al. 2013).

Because of this unique unilateral parasympathetic innervation of the pulmonary artery, *C. durissus* represents a suitable animal model to investigate whether the ability to generate R-L shunts enables metabolic rate to be reduced during prolonged fasting and hence confers prolonged survivorship that may have been favored by natural selection. The aim of this study was to investigate the hypothesis that the ability to reduce pulmonary blood flow and hence control the R-L cardiac shunt plays an important role in the regulation of metabolic rate in response to acute or long-term changes in metabolic demand. According to this hypothesis, removal of the ability to control the R-L cardiac shunt should affect the changes in metabolic rate induced by factors encountered by snakes, such as long-term food deprivation, fluctuating temperatures, or activity.

Material and Methods

Experimental Animals

This study utilized 21 juvenile (30 ± 4 g initial body mass) South American rattlesnakes (*Crotalus durissus*), born and raised in captivity at Universidade Estadual Paulista (Rio Claro, Sao Paulo, Brazil), and 18 adult rattlesnakes ($1,393 \pm 120$ g), acquired from the Butantan Institute (Sao Paulo). They were maintained individually in plastic boxes (40 cm × 29 cm × 27 cm) at 28°–30°C with ad lib. access to water and fed once a week until the beginning of the food deprivation protocol. All experiments were approved by the ethical committee on animal experimentation (Comissão de Ética na Experimentação Animal) at the Universidade Estadual Paulista (Botucatu, Sao Paulo, Brazil).

Unilateral Vagotomy

The experiments were performed on three groups of animals: sham-operated but otherwise intact (I) snakes and snakes with unilateral vagotomy on the left (LV) or on the right (RV) sides, as described by Leite et al. (2013). For induction of anesthesia, each snake was placed in a closed plastic chamber and exposed to elevated levels of CO_2 until reflexes disappeared (see Wang et al. 1993). Use of this procedure was discussed by Taylor et al. (2009). In the present experiments, it was a preliminary to intubation of the trachea and mechanical ventilation with 1%–4% isofluorane in air. A local anesthetic (2% lidocaine, Pearson) was injected subcutaneously, and the cervical vagi were exposed on either side through a 2–3-cm incision. The nerve was sectioned unilaterally, with a 1–2-cm segment of nerve removed to reduce the possibility of nerve regeneration. Because the left vagus alone controls resistance in the pulmonary artery (Taylor et al. 2009), the LV snakes were unable to control the cardiac

shunt after prolonged recovery (Leite et al. 2013). The RV group served as a control for any effect of unilateral vagotomy per se, while the sham-operated, intact group (I) served as a control for the operative procedures. The effectiveness and persistence of unilateral vagotomy were verified at the end of each experiment by recording the effect of peripheral stimulation of either vagus nerve (central to the point of transection) on heart rate and pulmonary and systemic blood flows (see Leite et al. 2013). The hemodynamic effect of unilateral left vagotomy was shown to be persistent over time, allowing long-term experiments (Leite et al. 2013).

Measurements of Metabolic Rate as Oxygen Uptake

RMR was determined in adult as well as juvenile snakes during their first year of development from a 30-h recording of the rate of oxygen uptake ($\dot{V}_{O_2}$), using a computer-controlled stop-flow respirometer (Sable Systems). Each respirometer was automatically closed for 10 min every hour while the decline in oxygen concentration within the chamber was measured by an oxygen analyzer (Sable Systems, PA-10) sampling at 1 Hz. During the subsequent open phase, air was renewed at 200 mL min^{-1}. The fall in oxygen concentration during the closed phase was fitted by a linear regression to calculate $\dot{V}_{O_2}$ (data were accepted only when the r^2 for the slope of the decline over 600 samples was more than 0.9). The lowest stable $\dot{V}_{O_2}$ at each temperature was taken as RMR. The RMR was recorded at 30° and 20°C during prolonged fasting and following reductions in temperature from 30° to 20° or 15°C in juvenile snakes during development.

During each set of 30-h measurements, snakes underwent spontaneous increases in activity levels so that recordings of $\dot{V}_{O_2}$ showed sporadically elevated values. The highest $\dot{V}_{O_2}$ values recorded were designated as $\dot{V}_{O_2}$spont and compared with RMR as an index of the ability of each snake voluntarily to raise its rate of aerobic activity, both with and without the ability to control the shunt. $\dot{V}_{O_2}$ was also measured in each snake following 5 min of forced activity at 20° and 30°C. Activity was imposed by moving/rotating the respirometer, forcing the snake to move continuously to adjust body position. The snakes were unable to maintain this level of activity for more than 5 min, at which point they were no longer able to perform righting movements, indicating that they were fatigued. This maximal $\dot{V}_{O_2}$ induced by forced activity was termed $\dot{V}_{O_2}$act and used as an indicator of the capacity of the snakes to increase metabolic rate when impelled to move, as described by Leite et al. (2013).

Fasting

To study the possible role of the cardiac shunt for metabolic regulation and allocation of energy resources, adult rattlesnakes were fasted under conditions similar to the holding conditions described above. Rattlesnakes can resist several months of fasting in laboratory conditions (Martin and Bagby 1973; McCue 2007, 2008). Food deprivation was maintained until the snakes had lost 30% of their body mass (possibly marking the begin-

ning of phase 3 of starvation; see Wang et al 2006). The snakes took 12 mo to lose 30% of their body mass. During that time, body mass was recorded each month during the first 4 mo and then every 2 mo. The South American rattlesnake normally experiences prolonged fasting in its natural environment, since the presence of suitable prey can be severely reduced or even absent during prolonged dry seasons, which can extend across calendar years (Ab Saber 1970; Greene 1983; Beaupre and Duvall 1998). They have been reported to survive more than 1 yr in their natural environment (A. S. Abe and W. Fernandes, personal communication).

During the period of food deprivation, RMR was measured as $\dot{V}O_2$ for each experimental group at 30° and 20°C, as described above. Measurements were made each month until the fourth month of fasting and then at bimonthly intervals until 12 mo. The $\dot{V}O_2$spont was also recorded at each interval, and $\dot{V}O_2$act was measured before and at the end of fasting to access any alteration on the scope for spontaneous or forced aerobic activity.

Temperature

To test the possible role of the cardiac shunt in changes of metabolic rate with temperature, we recorded the RMR as oxygen uptake over a 30-h period on inactive and undisturbed snakes from each experimental group, after a change in temperature. The animals were held at 30°C (temperature of acclimation) and then exposed to reductions in temperature to 20° or 15°C, with subsequent increases in temperature over the same range. The temperatures used are within the range of temperatures encountered by this species in its natural environment. The sequence of exposure to each temperature was randomized. The Q_{10} was calculated from individual values of RMR measured between 30° and 20°C and between 20° and 15°C. The effect of exposure to these 3 temperatures was recorded throughout the development of the three experimental groups of juvenile snakes at 4, 6, 10, and 13 mo and once in the adult snakes. The data obtained from juvenile snakes held at 30°C were previously presented by Leite et al. (2013).

Statistical Analyses

ANOVA two-way and Student-Newman-Keuls tests were used to identify significant effects of temperature or denervation on RMR, $\dot{V}O_2$spont, and $\dot{V}O_2$act and on absolute and relative reductions in body mass. A significance level was assigned in all cases ($P < 0.05$).

Results

Fasting

Food deprivation caused a gradual and slow reduction of body mass in all 3 experimental groups of adult snakes: intact snakes (group I), right vagotomized snakes (group RV), and left vagotomized snakes (group LV), which were unable to control the cardiac shunt. Over the full 12 mo of fasting, body mass

was reduced from 1,335 ± 102 to 999 ± 40 g (I), 1,280 ± 96 to 860 ± 108 g (LV), and 1,559 ± 42 to 1,069 ± 17 g (RV; fig. 1). After an initial loss during the first 2 mo of food deprivation, body mass was slightly reduced (from 91% to 89%, 83% to 81%, and 83% to 82% of the initial body mass in the intact, RV, and LV groups, respectively) during the next 6 mo. After that period of time, body mass declined fast during 8–12 mo of food deprivation (fig. 1A). There was no difference in the rate of mass loss between the groups (fig. 1). The relative loss in mass (fig. 1B) of each group was used to assess the metabolic phases (phases I, II, and II) of starvation. All groups changed the identified fasting phase at the same time. Phase II was reached at 2–3 mo, and phase III was reached at 9 mo of food deprivation. All snakes survived this protocol.

Long-term food deprivation did not reduce RMR in adult rattlesnakes in any of the three experimental groups throughout the entire year of food deprivation (fig. 2). Mean RMRs, recorded as $\dot{V}O_2$ (mL O_2 kg^{-1} h^{-1}), during fasting were 36.0 at
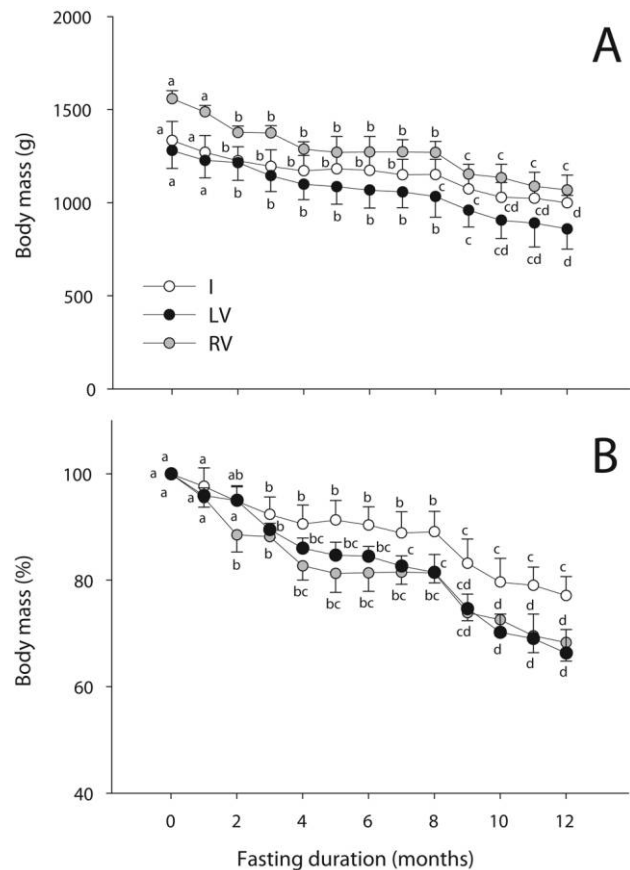

Figure 1. Progressive reduction in body mass (g; A) and relative mass (%; B) with time during food deprivation in the three groups of adult rattlesnakes (*Crotalus durissus*): intact (sham operated; I; white circles), right vagotomized (RV; gray circles), and left vagotomized (LV; black circles). All data are presented as means ± SEM. Different letters denote statistically significant differences between months. There were no differences between the groups (ANOVA two-way and Student-Neuman-Keuls, $P < 0.05$).

30°C and 12.3 at 20°C in the intact group, 35.3 at 30°C and 12.7 at 20°C in the RV group, and 37.3 at 30°C and 13.3 at 20°C in the LV group.

Besides maintaining RMR, the snakes retained the ability to increase metabolic rate. Spontaneous bursts of increased activity were observed in all snakes during every 30-h respirometry trial at both 30° and 20°C (fig. 3). There was no reduction in the maximal $\dot{V}O_2$spont throughout the period of food deprivation, and it was statistically similar between the experimental groups (fig. 4). Mean $\dot{V}O_2$spont, recorded as oxygen uptake (mL O_2 kg^{-1} h^{-1}), during fasting were 82.8 at 30°C and 22.5 at 20°C in the intact group, 88.8 at 30°C and 22.3 at 20°C in the RV group, and 104.8 at 30°C and 30.8 at 20°C in the LV group. Furthermore, the induced rates of activity at the outset and at the conclusion of the experiment resulted in elevated rates of oxygen uptake that were significantly higher than $\dot{V}O_2$spont and about 5 times higher than RMR for all the groups. There were no differences between mean $\dot{V}O_2$act recorded before and after 1 yr of food deprivation (fig. 4). Mean $\dot{V}O_2$act, recorded as oxygen uptake (mL O_2 kg^{-1} h^{-1}) before and after fasting, were 185.1 at 30°C and 103.7 at 20°C in the intact group, 179.7 at 30°C and 110.5 at 20°C in the RV group, and 178.3 at 30°C and 114.6 at 20°C in the LV group. Therefore, the metabolic scopes for increased rates of both spontaneous and forced activity did not change during prolonged fasting. There were also no significant differences between mean values of $\dot{V}O_2$spont and $\dot{V}O_2$act between experimental groups.

Temperature Change

RMR of juvenile snakes increased during development, peaking at 6 mo, when it was higher than the RMR of adults. RMR then decreased, reaching the adult level in 1-yr-old snakes. These changes in RMR were similar in all 3 experimental groups of juvenile snakes. The RMR of juvenile snakes held at an acclimation temperature of 30°C was reduced by 65% at 20°C and by 80% at 15°C and at all stages of maturation. Consequently, the calculated Q_{10} for RMR did not differ with age (table 1). Although RMR varied with temperature, there was no difference between the $\dot{V}O_2$ of snakes from all three groups at 30°, 20°, or 15°C (table 1). Thus, the ablation of shunt control did not affect the metabolic response to temperature change of juvenile snakes at all stages of early growth and development.

Discussion

Experimental Model

The LV rattlesnake has been validated as a model for short- and long-term studies on the functional role of the cardiac shunt (Taylor et al. 2009; Leite et al. 2013). This model provides an experimental group of reptiles that is unable to control the resistance of the pulmonary artery, and so it exhibits a constant high rate of perfusion of the lung circulation and an incapability to make significant adjustments to the R-L shunt. Consequently, this experimental model enables the supposed role of R-L shunting in controlling metabolic rate to be examined.
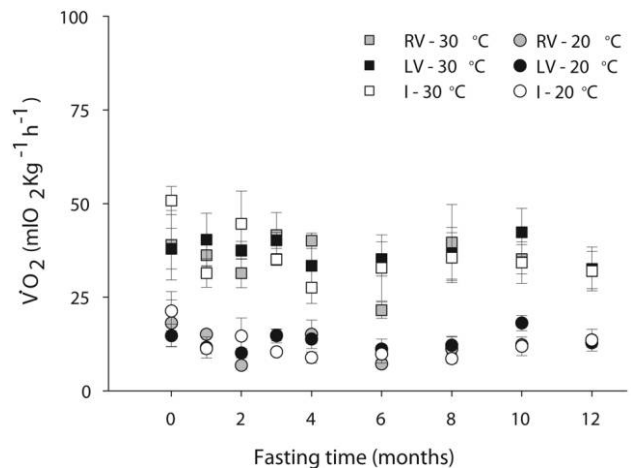

Figure 2. Resting metabolic rate recorded as oxygen uptake ($\dot{V}O_2$) at 30°C (squares) and 20°C (circles) over 12 mo of food deprivation in three groups of adult rattlesnakes (*Crotalus durissus*): intact (sham operated; I; white symbols), right vagotomized (RV; gray symbols), and left vagotomized (LV; black symbols). All data are presented as means ± SEM. There were no differences among the groups at each month or with duration of exposure to each temperature (ANOVA two-way and Student-Neuman-Keuls, $P < 0.05$).

In addition, snakes that were right vagotomized were indistinguishable from intact sham-operated snakes, being similar in the rate at which they lost body mass during fasting, their RMR before or during fasting at 20° or 30°C, and also their ability to increase metabolic rate during spontaneous or forced activity both before and after fasting at 20° or 30°C. Therefore, unilateral vagotomy per se was without effect on metabolic rate. The possibility of reinnervation of the pulmonary artery and restoration of the R-L shunt in denervated snakes during these long-term studies was eliminated in a previous investigation by measuring blood flow in snakes at the end of experimental protocols and by peripheral stimulation of the sectioned nerves (Leite et al. 2013).

Fasting

The rattlesnakes used in these experiments exhibited a progressive loss of mass during 1 yr of food deprivation without exhibiting external signs of deterioration in condition or responsiveness. We identified three distinct phases on the rates of reduction in body mass in all experimental groups. The first phase was a small reduction in body mass during the first 3 mo. This was followed by 6 mo of relatively stable body mass (very small reduction rate), followed by a phase of relatively rapid weight loss. Once this third phase was clearly identified, the experiment was terminated. All snakes survived the period of fasting. Although we did not collect biochemical data to confirm the metabolic relationship, this profile of weight loss during food deprivation resembles the three phases described for mammals, which refers to the preferential burning of specific metabolic reserves: carbohydrates, lipids, and proteins, respectively, in phases I, II, and
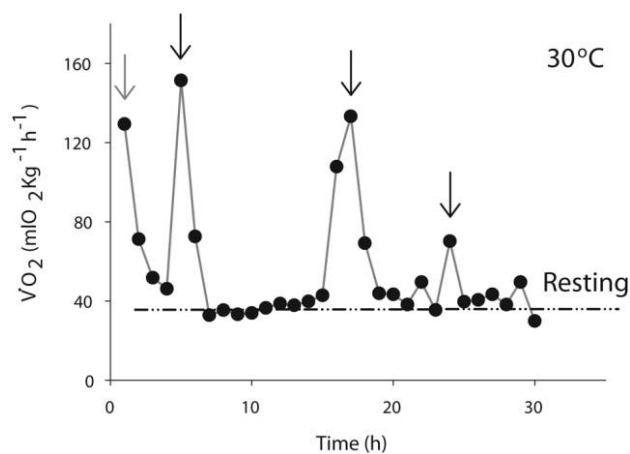
Figure 3. Representative recording of 30 h of intermittent closed respirometry in *Crotalus durissus* at 30°C. Each point is taken from a period of closed respirometry. The baseline (horizontal line) was taken as the resting metabolic rate (38.04 mL O_2 kg^{-1} h^{-1}). The gray arrow indicates the effect of handling on initial $\dot{V}O_2$. Black arrows denote increases in $\dot{V}O_2$ due to spontaneous activity.

III (Wang et al. 2006). However, those phases were comparatively much longer if compared with any mammalian species, since the snakes took 8 mo to reach phase III. That is to be expected, since the relatively low RMR of rattlesnakes would reduce their rates of depletion of energy reserves.

Rattlesnakes (*Crotalus atrox*) exposed to food deprivation showed reduced plasma glucose levels together with increased concentrations of ketone bodies, indicating lipid mobilization. After the first 3 mo, the concentrations of these components stabilized (McCue 2007). Lipid mobilization may account for the relatively slow rate of weight loss after the third month of food deprivation, denoting the switch to phase II, as described for mammals. The adult snakes used in the present experiments were three times bigger than the ones used by McCue (2007) and may have had a larger amount of stored energy as fat, accounting for their longer phase II.

Survival during prolonged periods of fasting may rely on reduction of metabolic rate. In general, metabolic rate can be reduced by suppressing reproduction, reducing activity, or lowering general metabolism. That is true for mammals that have rapidly depressed their metabolism when facing food deprivation (Keys et al. 1950). The South American rattlesnake is a reptilian species able to survive long periods of food deprivation in its natural environment. The Brazilian biomes of Caatinga and Cerrado can suffer drought conditions for much of the year with a consequent fluctuation in the abundance/scarcity of prey (Ab Saber 1970).

Cardiac R-L shunt has been shown to be inversely correlated with metabolic rate (Mitchell et al. 1981a, 1981b; Burggren 1987), so the cardiac shunt was suggested to play a role in regulating metabolic depression during food deprivation (Wang et al. 2006). In the turtle *Trachemys scripta*, experimental reduction of pulmonary blood flow ($\dot{Q}$pul), with the consequent

reduction in systemic oxygen levels, was demonstrated to trigger hypometabolism. The hypometabolic state was overcome by injection of 2,4-dinitrophenol, demonstrating that metabolism was somehow inhibited by—and not limited by—the reduction of the oxygen delivery to the tissues (Hicks and Wang 1999; Platzack and Hicks 2001). Hence, the hypometabolism induced by tissue hypoxia due to an increase in the net R-L cardiac shunt has been presented as a putative potent resource enabling the long diving duration in turtles (Hicks and Wang 2004). The possibility that tissue hypoxia can trigger a hypometabolic state at the cellular level is relevant to consideration of the present data. Changes in the relative perfusion of the
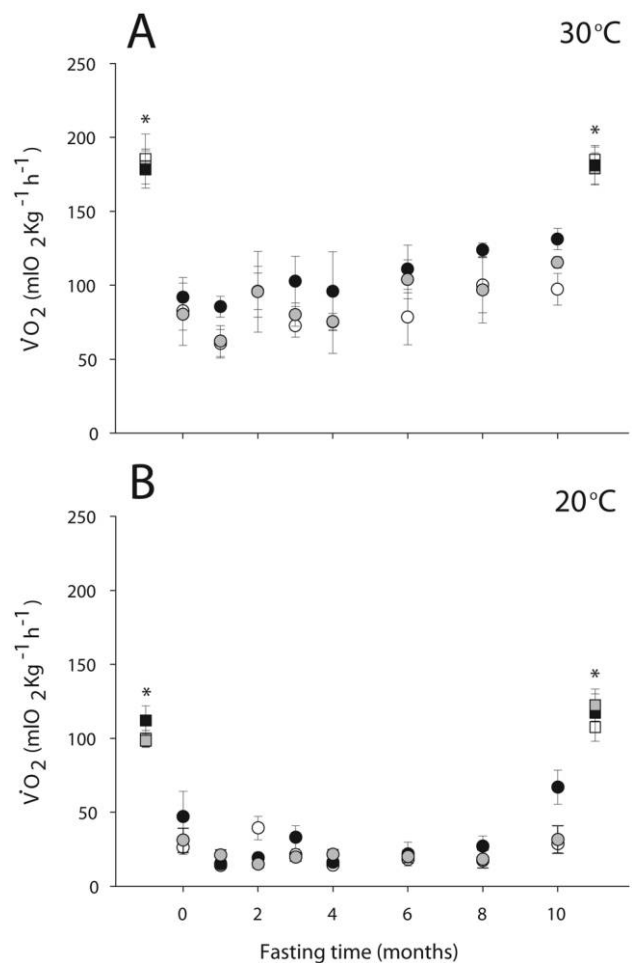

Figure 4. Metabolic rate recorded as oxygen uptake ($\dot{V}O_2$; mL O_2 kg^{-1} h^{-1}) at spontaneous ($\dot{V}O_2$spont; circles) and forced ($\dot{V}O_2$act; squares) levels of increased activity at 30°C (*A*) and 20°C (*B*) in three groups of *Crotalus durissus*: intact (sham operated; I; white circles), right vagotomized (RV; gray circles), and left vagotomized (LV; black circles). Spontaneous increases in $\dot{V}O_2$ were recorded during the period of food deprivation, while the $\dot{V}O_2$act was recorded immediately before and at the end of the period of prolonged fasting. Points are means ± SEM. There were no differences between groups at any point in the experiment. Asterisks denote significant differences between the metabolic rates measured during spontaneous and forced activity (ANOVA two-way and Student-Neuman-Keuls, $P < 0.05$).

Table 1: Resting metabolic rate at three different temperatures recorded in three groups of rattlesnakes (*Crotalus durissus*)

Age and group	n	Mass (g)	30°C	Q_{10} 30°–20°C	20°C	Q_{10} 20°–15°C	15°C
4 mo:							
I	7	52.3 ± 4.1	49.50 ± 5.22[A]	2.9 ± .1	17.02 ± 3.37[B]	2.7 ± .1	11.72 ± 1.43[C]
LV	7	52.1 ± 2.8	46.87 ± 2.35[A]	2.9 ± .1	18.32 ± 1.47[B]	2.9 ± .2	10.84 ± .79[C]
RV	7	49.0 ± 3.4	49.52 ± 4.57[A]	2.9 ± .2	17.19 ± 1.28[B]	3.1 ± .5	9.53 ± 1.36[C]
6 mo:							
I	7	66.0 ± 8.5	66.34 ± 2.35[A*]	2.2 ± .1	30.13 ± 2.07[B*]	1.9 ± .1	20.99 ± 2.19[C*]
LV	7	89.5 ± 9.6	59.89 ± 3.35[A*]	2.4 ± .1	25.00 ± 3.37[B*]	2.4 ± 0.2	15.78 ± .91[C*]
RV	7	81.3 ± 12.7	63.69 ± 6.99[A*]	2.2 ± .2	29.04 ± 4.11[B*]	2.0 ± .3	21.13 ± 5.19[C*]
10 mo:							
I	7	221.7 ± 28.5	52.99 ± 3.20[A]	2.8 ± .2	18.69 ± 1.70[B]	2.51 ± 0.2	11.46 ± 1.54[C]
LV	7	238.0 ± 20.3	48.09 ± 3.51[A]	2.9 ± .2	16.63 ± 1.64[B]	2.2 ± .5	11.20 ± .99[C]
RV	7	204.16 ± 39.6	53.67 ± 5.25[A]	2.9 ± .2	17.12 ± 1.18[B]	2.3 ± .5	11.58 ± 1.00[C]
13 mo:							
I	7	285.1 ± 25.9	41.47 ± 3.45[A]	3.2 ± .2	13.09 ± 1.33[B]	3.1 ± .3	7.41 ± .67[C]
LV	7	323.7 ± 13.9	45.40 ± 5.94[A]	3.3 ± .1	11.97 ± 1.63[B]	3.3 ± .2	6.50 ± .67[C]
RV	7	256.4 ± 33.6	44.89 ± 4.77[A]	3.1 ± .1	14.37 ± 1.83[B]	3.3 ± .2	7.11 ± .55[C]
Adult:							
I	6	1059.3 ± 143.9	38.31 ± 5.89[A]	2.4 ± .2	16.36 ± 3.36[B]	2.6 ± .4	10.27 ± 3.07[C]
LV	6	2136.4 ± 162.4	35.27 ± 6.30[A]	2.7 ± .1	12.95 ± 2.34[B]	2.5 ± .5	8.86 ± 2.46[C]
RV	6	1954.1 ± 203.8	37.01 ± 8.30[A]	2.7 ± .2	13.76 ± 1.22[B]	2.1 ± .4	9.97 ± 2.99[C]

Note. I, intact (sham operated); LV, left vagotomized; RV, right vagotomized. Differences between mean rates are reported as Q_{10} values. Resting $\dot{V}_{O_2}$ (mL O_2 kg^{-1} h^{-1}) was recorded in juvenile snakes during development (4, 6, 10, and 13 mo) and in adult snakes. Data from juvenile snakes at 30°C were obtained from the study by Leite et al (2013). Data are means ± SE. Different letters denote both intragroup (temperature) and intergroup (treatment) differences (two-way ANOVA for repeated measures and Student-Newman-Keuls, $P < 0.05$).

*Denotes higher resting metabolic rate during development.

pulmonary circuit have been shown to have strong proportional effects on arterial blood oxygen content (Wang and Hicks 1996, 2002; Wang et al. 1997), altering the oxygen cascade (Wang and Hicks 2002; Taylor et al. 2009). So reduced metabolic rates would enable survival of prolonged fasting. Nevertheless, the present data show that control of the cardiac shunt was not important for determining metabolic regulation of RMR or long-term energy usage. The LV rattlesnakes were unable to reduce pulmonary blood flow or the net shunt (i.e., the $\dot{Q}$pul/$\dot{Q}$sys ratio). We suggested that any subtle metabolic alterations caused by R-L shunt would be likely to have resulted in observable changes in the mass loss curve over the prolonged fasting times experienced by the snakes in the present experiments. Each of the experimental groups changed the identified fasting phase at the same time, so the rate of energy mobilization and depletion of energetic reserves were not affected by ablation of the cardiac shunt. In addition, the snakes did not reduce the capacity to mobilize energy for activity during the fasting period. The rate of oxygen uptake for spontaneous or forced activity recorded throughout the period of food deprivation compares with the prefasting level, so the reduction of metabolic cost based on decreasing the metabolic scope for activity was not observed in rattlesnakes. The ability of snakes to increase $\dot{V}_{O_2}$ to $\dot{V}_{O_2}$act when disturbed at the end of a prolonged period of fasting may be important for the processing of the first meal at the end of the fast. It is been reported that some reptiles attain higher rates of oxygen consumption during the postprandial period after ingestion of large meals compared with physical activity (Bennett and Hicks 2001; Hicks and Bennett 2004). This was the case in the rattlesnake that has a specific dynamic action peak (after the ingestion of the equivalent to 30% of its body mass at 30°C) two times higher than the present reported $\dot{V}_{O_2}$act (Leite et al. 2013). The $\dot{V}_{O_2}$act in this case does not represent the maximum metabolic rate the snake can reach but may represent the capacity for aerobic recovery from fatigue that can arise from a bout of enforced, possibly anaerobic activity. Similar brief bouts of activity may be the prelude to ingestion and the onset of specific dynamic action while capturing a prey at the end of a period of food deprivation (Wang et al. 2001). The fact that the rattlesnakes, unable to operate the R-L shunt, showed no alterations in measured variables such as the slope of the mass loss curves and duration of fasting phases, oxygen consumption during RMR or activity, and their metabolic scope for both spontaneous and forced activity throughout the months of food deprivation provides strong evidence that the cardiac shunt does not have an important role in the metabolic adjustments enabling survival of prolonged food deprivation.

Temperature

As expected for an ectothermic species, metabolic rate in the rattlesnake was strongly affected by changing experimental temperature. On the basis of the blood flow measurements reported

by Leite et al. (2013), the intact rattlesnake would maintain a net R-L shunt while at rest at a range of temperatures. Due to the lack of control of the pulmonary artery constriction, $\dot{Q}$pul was increased in the LV group at 15°, 20°, and 30°C, the same temperatures used in the present experiment. The ratio $\dot{Q}$pul/$\dot{Q}$sys was higher in LV snakes and increased as temperature decreased, resulting in a net L-R shunt at 15°C (Leite et al. 2013). The expected consequent effect on the arterial blood gases and oxygen cascade did not have any measurable effects on RMR in the present experiment.

The absence of functional significance of the cardiac shunt on metabolic adjustment was observed in freely diving turtles (*T. scripta*) subjected to partial occlusion of the pulmonary artery. The consequent impairment of pulmonary blood flow and the ability to increase it in response to increased activity levels had no effect on the rate of gas exchange. Neither oxygen uptake nor CO_2 excretion correlated with changes in pulmonary blood flow (Wang and Hicks 2008). The authors suggested that the increases in pulmonary blood flow associated with ventilation are not required to maintain RMR and normal CO_2 excretion in resting turtles. Rates of oxygen uptake in juvenile rattlesnakes changed with maturation, increasing at 6 mo post-hatching—possibly because of an increased energy demand for growth—and then decreasing to adult levels by 10–13 mo. The increased RMR during maturation, its timing, and duration were the same between the 3 experimental groups. Temperature change had the same effect on the RMR of the three groups; therefore, control of the cardiac shunt appears to be unimportant in generating metabolic responses to temperature change during growth and maturation.

Conclusions

Selective vagotomy of the rattlesnake (*Crotalus durissus*) provides an experimental model to investigate the role of the R-L cardiac shunt in the provision of metabolic adjustments. This investigation revealed that the cardiac shunt does not provide any fundamental advantage or play an important role in (1) the maintenance of RMR of food-deprived *C. durissus* at 20° and 30°C; (2) the sequential usage of stored energy during long-term food deprivation; (3) the scope for energetic mobilization during spontaneous and forced activity, even in animals facing long-term food deprivation; and (4) the maintenance of RMR at 15°, 20°, and 30°C, including during the development of juvenile rattlesnakes. Consideration of the present data, together with the previously reported work of Leite et al. (2013), encourages rejection of the hypothesis that controlling the degree of mixing of blood returning from the pulmonary and systemic circuits in the undivided ventricle of reptiles has a functional significance in the control of metabolic rate. Our findings are consistent with recent studies on crocodilians where surgical occlusion of the left aortic arch also provide an experimental manipulation to characterize the functional consequences of eliminating the possibility of R-L shunts (Eme et al. 2009, 2010; Hicks and Wang 2012). Thus, the evidence currently available from both acute and long-term studies on

both rattlesnakes and crocodilians does not support the notion of adaptive advantage provided by the ability to shunt oxygen poor blood back to the systemic circulation.

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