

Role of the post-hepatic septum on breathing during locomotion in *Tupinambis meriana* (Reptilia: Teiidae)

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Summary

Tupinambis meriana increased minute ventilation by increasing both tidal volume and breathing frequency during sustained locomotion at 0.17 m s^{-1} . Animals in which the post-hepatic septum (PHS) had been surgically removed were not able to increase tidal volume during locomotion. Tegus without PHS compensated, in part, by increasing breathing frequency above the levels observed for tegus with intact PHS, but minute ventilation remained less than in the control animals. The rate of oxygen consumption and the air convection requirement,

however, were not significantly different between animals with and without PHS, nor at the tested speeds was endurance affected by the removal of the PHS. These data suggest that the PHS facilitates ventilation by acting as a mechanical barrier, preventing the viscera from moving cranially during physical exertion.

Key words: *Tupinambis meriana*, lizard, post-hepatic septum, locomotion, ventilation, breathing mechanics.

Introduction

The effects of locomotion on breathing in lizards have been the subject of numerous studies (Carrier, 1987a; Cragg, 1978; Farmer and Hicks, 2000; Hopkins et al., 1995; Mitchell et al., 1981; Owerkowicz et al., 1999; Wang et al., 1997; Wood et al., 1978). Most studies have been performed on the savannah monitor *Varanus exanthematicus* and on the green iguana *Iguana iguana*. These two species belong to phylogenetically distinct groups of lizards that differ markedly in their ecology, *V. exanthematicus* being a cursorial forager and *I. iguana* an arboreal herbivore. Both species also differ significantly in lung morphology. Varanid lizards possess large, multichambered lungs, which may occupy up to two thirds of the body cavity and are separated from the rest of the viscera by a post-pulmonary septum, whereas the smaller, paucicameral lungs of Iguanids lie in an undivided pleuro-peritoneal cavity (Duncker, 1978). In contrast to *I. iguana*, *V. exanthematicus* uses gular pumping during exercise and is able to increase minute ventilation with increasing speed up to its maximum minute ventilation (Owerkowicz et al., 1999). Gular pumping also helps varanids to compensate the 'axial constraint' that may prevent lizards from breathing while running at high speeds (Carrier, 1987a,b; Wang et al., 1997).

Teiid lizards of the genus *Tupinambis* are ecologically similar to the savannah monitor but their lungs and pattern of internal septation are fundamentally different. They possess single-chambered lungs with homogeneously distributed parenchyma (Duncker, 1978; Perry, 1983). Teiids are also characterised by a well-developed post-hepatic septum (PHS)

(Broman, 1904; Duncker, 1978), which incompletely divides the body cavity into two parts. The cranial part contains the liver and the lungs, which are fixed in the body cavity by dorsal mesopneumonia, while the caudal part contains the remaining viscera (Klein et al., 2000). The morphology of the PHS varies in teiid lizards. It is best developed, almost completely closed, in *Crocodylus* and *Tupinambis*, whereas the PHS in smaller teiids, such as *Ameiva* and *Cnemidophorus*, only attaches to the ventro-lateral part of the body wall (Klein et al., 2000).

Morphological diffusing capacity has been shown to be similar in the savannah monitor and the tegu, *T. teguixin* (earlier *T. nigropunctatus*; Avila-Pires, 1995) (Perry, 1983) but ventilation of tegus has not yet been measured during exercise or interpreted in terms of breathing strategies. Furthermore, the deep, densely partitioned lung parenchyma of tegu lungs is well suited for a sedentary life-style of a sit-and-wait predator, which does not need to support a high rate of oxygen consumption for prolonged periods (Perry, 1998). An actively foraging life-style as in the savannah monitor, on the other hand, favours a convective gas-exchange strategy to provide high levels of gas exchange during activity (Perry, 1998). Accordingly, the savannah monitor shows a more shallow and less densely partitioned parenchyma, which can be ventilated with low work of breathing (Perry, 1983). In the tegu, also a highly active lizard, only the lung parenchyma directly exposed to the lung lumen is accessible for convective gas exchange, whereas gas exchange in the deep faveolar

parenchyma depends mainly on diffusion and contraction of smooth muscle to remove the used air. Furthermore, tegu lungs are of low compliance (Klein et al., 2003) and require a high work of breathing to ventilate them (Perry and Duncker, 1980). Given these differences between savannah monitors and tegus, the tegu lizard appears to demonstrate a mismatch between form and function, as its low-compliance, energy-expending respiratory system has several disadvantages for a lifestyle as an actively foraging predator.

Because the tegu shows the best developed PHS of all teiid lizards, we suspect that this septum may reduce the axial constraint to lung ventilation during exercise at moderate speeds and compensate for the limitations set by the diffusion-dominated lungs. This hypothesis was tested by measuring oxygen uptake and ventilatory parameters of tegus with and without post-hepatic septum on a motorised treadmill.

Materials and methods

Animals

All specimens of *Tupinambis meriana* Lacépède 1788 used in this study were captive-bred at the Jacarezário, Universidade Estadual Paulista in Rio Claro, São Paulo, in south-eastern Brazil. Tegus were housed in plastic boxes (74 cm×56 cm×41 cm), containing wood chips and kept on a 12:12 h L:D cycle. They were fed every third day with minced meat, to which bicalcium phosphate, salt, minerals and cornmeal were added. Water was provided *ad libitum*. Details of maintenance and breeding of tegus in Rio Claro were described by Lopes and Abe (1999).

Ventilation and gas exchange

Ventilation was studied using the mask technique of Glass et al. (1978), modified by Wang and Warburton (1995). For ventilation measurements during the night, a plastic mask enclosing the entire head and containing a pneumotachometer was employed. For the locomotion experiments the tip of the tegu's snout, including nares and closing the mouth tightly, was covered with a small plastic mask, which was connected to a pneumotachometer by a short piece of Tygon® tubing. In both cases the mask was connected by the tubing to a pressure transducer (Sable PT-100; Las Vegas, USA) and an O₂ analyser (Applied Electrochemistry S-3 A/L; Pittsburgh, USA). Flow through the mask was generated by a suction pump (Ametek, Paoli, USA; flow control R1) placed downstream from the O₂ analyser. To remove water vapour and CO₂ from the expired air, a tube containing silica gel and Ascarite® was placed upstream to the O₂ analyser. For ventilation measurements at night, flow rate was kept constant at 200 ml min⁻¹, whereas flow rate was 300 ml min⁻¹ for ventilation measurements on the treadmill. These flow rates allowed breath-by-breath analysis, although the signal from the oxygen analyser was delayed for 2–3 s. The signals from the pressure transducer and oxygen analyser were recorded through a computerised data-acquisition system (DAC, Sable System). To calibrate the masks, a plaster cast of a tegu's head

was made, placed into the mask and sealed with latex rubber. The masks were calibrated with simulated breaths of known volume and gas composition. The relationship between the electrical signal generated and volume and composition of gas could be accurately described by linear regressions ($r^2 > 0.9$ in all cases).

Surgery

Septum removed (SR)-tegas

To remove the PHS, tegus were anaesthetised with CO₂ (Wang et al., 1993). When the animal no longer reacted to pinching of the skin, it was fixed with rubber bands in a supine position on an operation table and a constant low CO₂-flow was provided to the nostrils. The belly was disinfected with tincture of iodine before surgery. A 1.5–2.5 cm incision was made on the ventro-lateral body wall caudal to the last long rib. The fat-body, stomach and intestine were retracted, the PHS was exposed and completely ruptured in a stepwise fashion beginning at the lateral part of the body wall and moving to the dorsal midline and ventrally, approaching the abdominal vein as close as possible. The caudal part of the hepatic ligament was also ruptured. A similar rupture of the PHS was also carried out on the other side of the animal. The cuts were closed with suture, disinfected with iodine and Baytril™ was injected intraperitoneally to prevent infections after the surgery. Tegus were then allowed to recover for at least 3 months before experimentation.

Sham-operated (SO)-tegas

The procedure for the sham-operated animals was the same as for the SR-tegas, except that the PHS was not ruptured.

Experimental protocol

Experiments were performed with 5 SR-tegas (body mass, 855±343 g; snout–vent length, 278±28 mm) and 5 SO-tegas (body mass, 712±234 g; snout–vent length, 266±28 mm) after they had been allowed to recover for at least 3 months following surgery. Ventilation of resting animals, determined during the inactive period at night, was determined 3 weeks before the treadmill experiments. At least 24 h before making the recordings, the masks were attached to the tegus, sealed around the neck with latex rubber and secured with adhesive tape. The tegus were then placed into a climatic chamber at 35°C and ventilatory parameters were recorded during the following night between 00:00 h and 04:00 h. Before experimentation, food was withheld for at least 4 days.

The masks for the treadmill experiment were fixed on the tegu's snout the day before the experiment and tegus were kept overnight in a climatic chamber at 35°C. The next day, the animal was placed on the treadmill and the mask was connected to the experimental set-up. Room temperature was 28–30°C and a lamp over the treadmill maintained a temperature of 35°C on the belt. The belt of this custom-made motorised treadmill was 65 cm×78 cm (width×length); a cardboard box frame was used to prevent the animal from escaping.

Treadmill protocol

After an acclimatisation period of approximately 5 min, tegus were made to walk at a belt speed of 0.17 m s^{-1} for 5 min. They were then left undisturbed for 5 min, made to locomote at 0.28 m s^{-1} until exhaustion and then left undisturbed for 30 min. Tegus were motivated to run by pinching or tapping the tail. Running experiments were stopped when tegus could no longer be motivated with 10 consecutive stimuli (at 1 s^{-1}). These speeds were chosen for experiments on sustained locomotion, because tegus only show sprints of high speeds and short duration when chased or attacked (W.K., personal observation). Freely ranging tegus maintained in the outdoor pens moved at moderate speeds and foraged for prolonged periods (W.K., personal observation). Furthermore the effect of the PHS is expected to be greater at elevated aerobic states rather than during anaerobic sprints.

Endurance

Test of locomotor endurance of SO- and SR-tegus were measured 2 weeks before ventilatory measurements. Tegus that were not wearing masks were made to walk on the treadmill at either 0.17 m s^{-1} or 0.28 m s^{-1} until exhaustion. Measurements were repeated the next day at the same speed. The animals were tested at the other speed on two consecutive days. The greatest value of each speed obtained for a given animal were used to determine its endurance.

Data handling and statistics

For the animals resting at night, a period of 5 min or at least 30 consecutive breaths was analysed. This period was chosen to reflect a regular steady-state breathing pattern under this condition. From the treadmill experiment the following parts of the breathing trace were analysed: (1) 1 min before starting exercise (pre-exercise), (2) the last minute of the exercise periods (0.17 m s^{-1} and 0.28 m s^{-1} , respectively), (3) the first and the last min of the recovery period following exercise at 0.17 m s^{-1} , and (4) for 1 min immediately after exercising at 0.28 m s^{-1} , and 5, 10, 20 and 30 min later.

In all cases, we determined breathing frequency (f_R), tidal volume (V_T), duration of total respiratory movements (T_{TOT}), oxygen uptake per breath (V_{BO_2}), and oxygen extraction coefficient (E_{O_2}). From these measurements the rate of oxygen consumption (\dot{V}_{O_2}), total ventilation rate (\dot{V}_E) and air convection requirement for O_2 (\dot{V}_E/\dot{V}_{O_2}) were calculated. Data were transformed to STPD conditions following Dejours (1981).

To detect differences between previously defined parts of the breathing trace, a one-way repeated-measures analysis of variance (ANOVA) was used, followed by a paired multiple comparison procedure (Student–Newman–Keuls) to identify the group or groups that differed from each other. SO- and SR-tegus were compared using a paired *t*-test. A difference was considered significant at a level of $P \leq 0.05$. If the test for normality failed, data were \log_{10} transformed and reanalysed.

Results

A sample recording of ventilation and oxygen consumption is given in Fig. 1 and the results for T_{TOT} , f_R , V_T , \dot{V}_E , V_{BO_2} , \dot{V}_{O_2} , \dot{V}_E/\dot{V}_{O_2} and E_{O_2} are shown in Figs 2 and 3.

Ventilation at night and day

In SO-tegus all ventilatory parameters investigated, except V_T , showed significant differences ($P \leq 0.05$) between the recordings during the night and during the day. This was also the case for most of the parameters investigated in SR-tegus, but in this group T_{TOT} and \dot{V}_{O_2} also showed no significant differences between day and night. In general the length of a respiratory cycle was longer during the night. In addition f_R , \dot{V}_E , \dot{V}_{O_2} and \dot{V}_E/\dot{V}_{O_2} were smaller during the night whereas V_{BO_2} and E_{O_2} were greater during the night than during the day.

Ventilation during the activity

T_{TOT} before exercise was 1.73 s for SO-tegus and 1.92 s for SR-tegus but decreased in both groups during exercise to the shortest T_{TOT} values recorded (Fig. 2A).

V_T in SO-tegus during pre-exercise was only significantly different from the values during walking at 0.17 m s^{-1} and in the first minute of recovery from either speed (Fig. 2C). V_T in SR-tegus showed no significant differences between rest and exercise at both speeds. Exercising at 0.17 m s^{-1} increased \dot{V}_E significantly ($P \leq 0.05$) compared with pre-exercise values, both in SO- and in SR-tegus (Fig. 2D). This change was due to a significant increase in V_T in SO-tegus and f_R in SR-individuals. At this speed SO- and SR-tegus also differed in f_R and V_T ($P=0.048$ and 0.0102 , respectively), but not in \dot{V}_E ($P=0.0621$). In the first minute after exercise at 0.17 m s^{-1} \dot{V}_E decreased significantly ($P \leq 0.05$). f_R also decreased to pre-exercise values in both groups, but V_T remained at the level reached during exercise (18.76 and 19.89 ml kg^{-1} , respectively) in SO-tegus. In SR-tegus V_T increased from 11.6 to 16.1 ml kg^{-1} . 5 min after exercising at 0.17 m s^{-1} , f_R , V_T and \dot{V}_E returned to pre-exercise values in both groups. At 0.28 m s^{-1} , f_R , V_T and \dot{V}_E increased significantly ($P \leq 0.05$) compared to the preceding resting phase, with the exception of SR-tegus, in which V_T was only slightly increased above the resting values. The high \dot{V}_E in SO-tegus was a result of an increase in V_T together with a small decrease in f_R . V_T 1 min after 0.28 m s^{-1} was significantly ($P=0.0117$) lower in SR-tegus than in SO-tegus.

As a result of exercise, E_{O_2} decreased when compared to daytime resting values (Fig. 3A). SO- and SR-tegus showed the lowest E_{O_2} during fast walking at 0.28 m s^{-1} and during the first minute of this recovery period and returned towards resting values 30 min after exercise. SO- and SR-tegus decreased V_{BO_2} during exercise compared with the preceding phase. The highest values for oxygen uptake per breath were reached in the first min after walking at 0.17 m s^{-1} in both groups. \dot{V}_{O_2} , on the other hand, was high both during exercising at 0.17 m s^{-1} and the following first minute of recovery (Fig. 3C). Only SO-tegus showed a tendency to increase V_{BO_2}

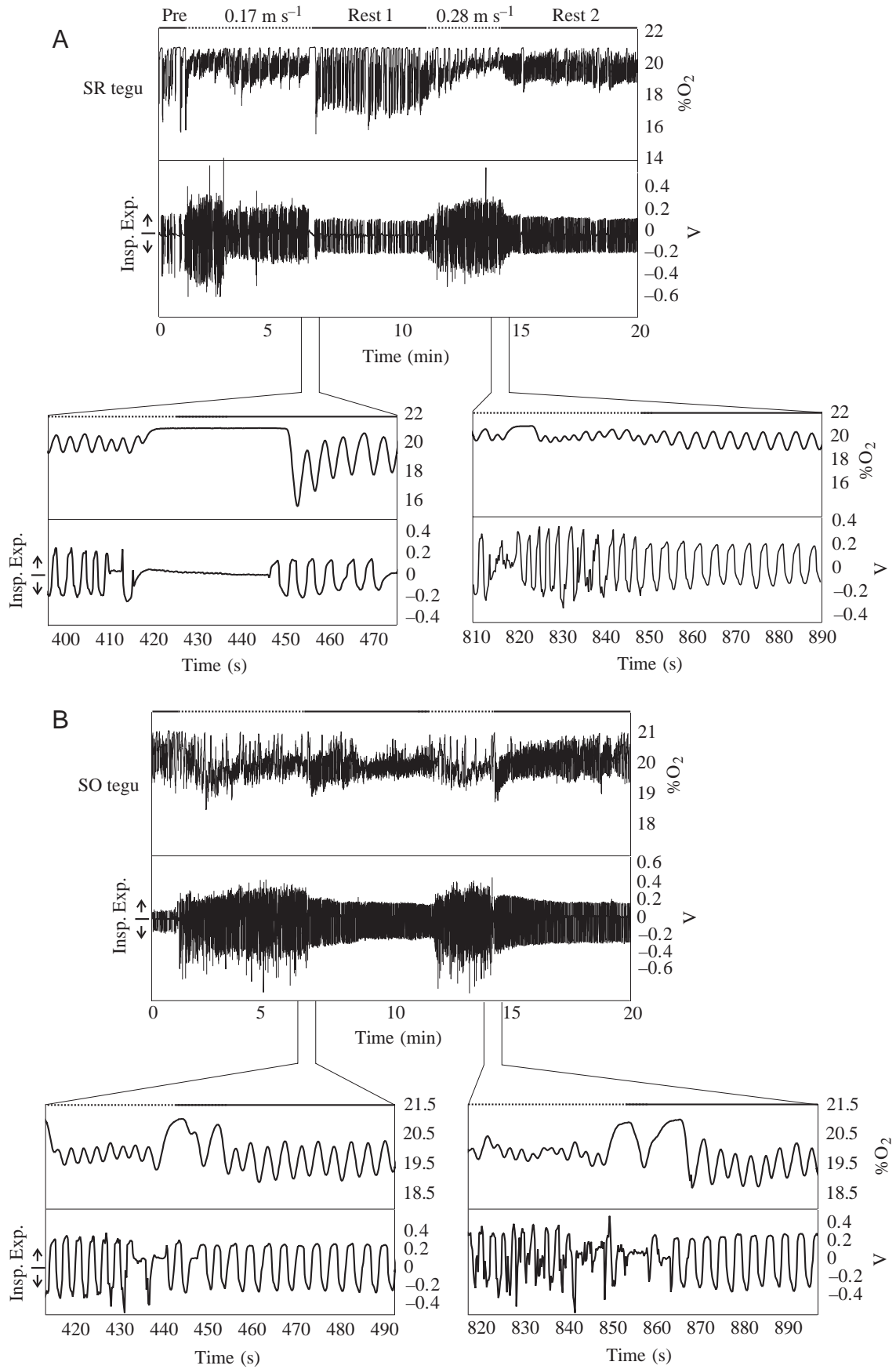


Fig. 1

Fig. 1. Sample recording of ventilation in tegus. (A) Septum removed (SR), (B) sham-operated (SO). The sequence contains pre-exercise (Pre), exercise at 0.17 m s^{-1} (0.17 m s^{-1}), 5 min of rest (Rest 1), exercise at 0.28 m s^{-1} (0.28 m s^{-1}) and the final recovery period (Rest 2). The transitions end of exercise to recovery period are expanded below each main trace. Upper traces, signal from oxygen analyser; lower traces, signal from pressure transducer. Above the ventilation trace a broken line indicates an exercise period and the solid line a resting period. Insp., inspiration; Exp., expiration.

and \dot{V}_{O_2} in the first minute after 0.28 m s^{-1} , which was not significantly different from the pre-exercise value.

\dot{V}_E/\dot{V}_{O_2} did not differ significantly between SO- and SR-tegus under any of the conditions investigated; however, \dot{V}_E/\dot{V}_{O_2} in SO-tegus was significantly different from the pre-exercise value during the first minute after exercising at 0.28 m s^{-1} . In addition, SO-tegus showed a significantly greater \dot{V}_E/\dot{V}_{O_2} at 0.28 m s^{-1} compared to the preceding recovery period.

Endurance

There was a large individual variation in the endurance of SO-tegus, ranging from 4.2 to 142.5 min at 0.17 m s^{-1} and from 2.3 to 70.3 min at 0.28 m s^{-1} . SR-tegus also showed a great variation at 0.17 m s^{-1} (3.5–109.7 min) and 0.28 m s^{-1} (1.5–17.9 min). Because of this great spread in values, no significant differences between group means for SO- and SR-tegus were found at either speed, but in both groups endurance at 0.28 m s^{-1} was significantly less than at 0.17 m s^{-1} (two-way repeated-measures ANOVA, $P \leq 0.05$). On average, however, SO-tegus could keep up with the lower belt speed for 47.6 min and the greater speed for 18.3 min, which was greater than in SR-tegus. Thus, endurance was 36.5 min at 0.17 m s^{-1} and 5.2 min at 0.28 m s^{-1} (Fig. 4).

Because of the large variation, the median value appeared the more attractive indicator of central tendency for this parameter. The median was greater in

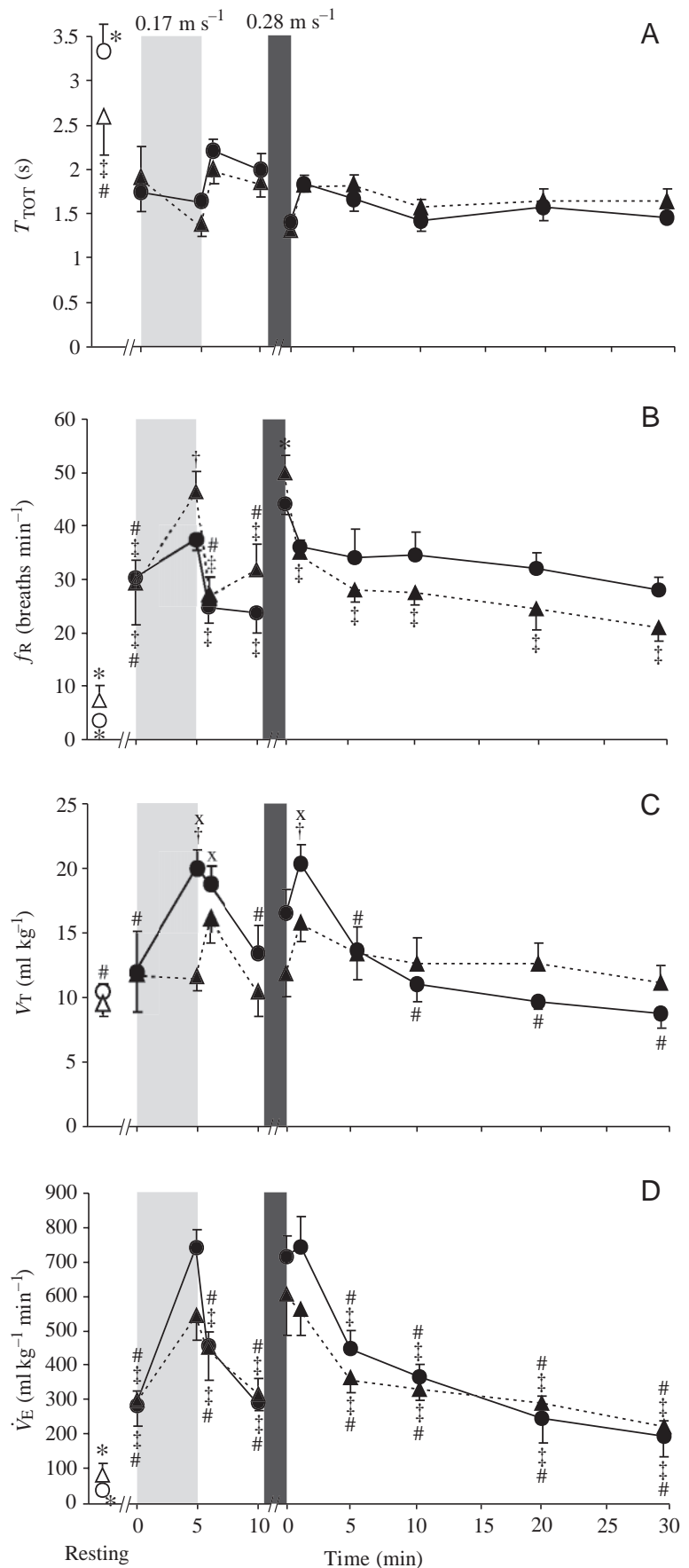
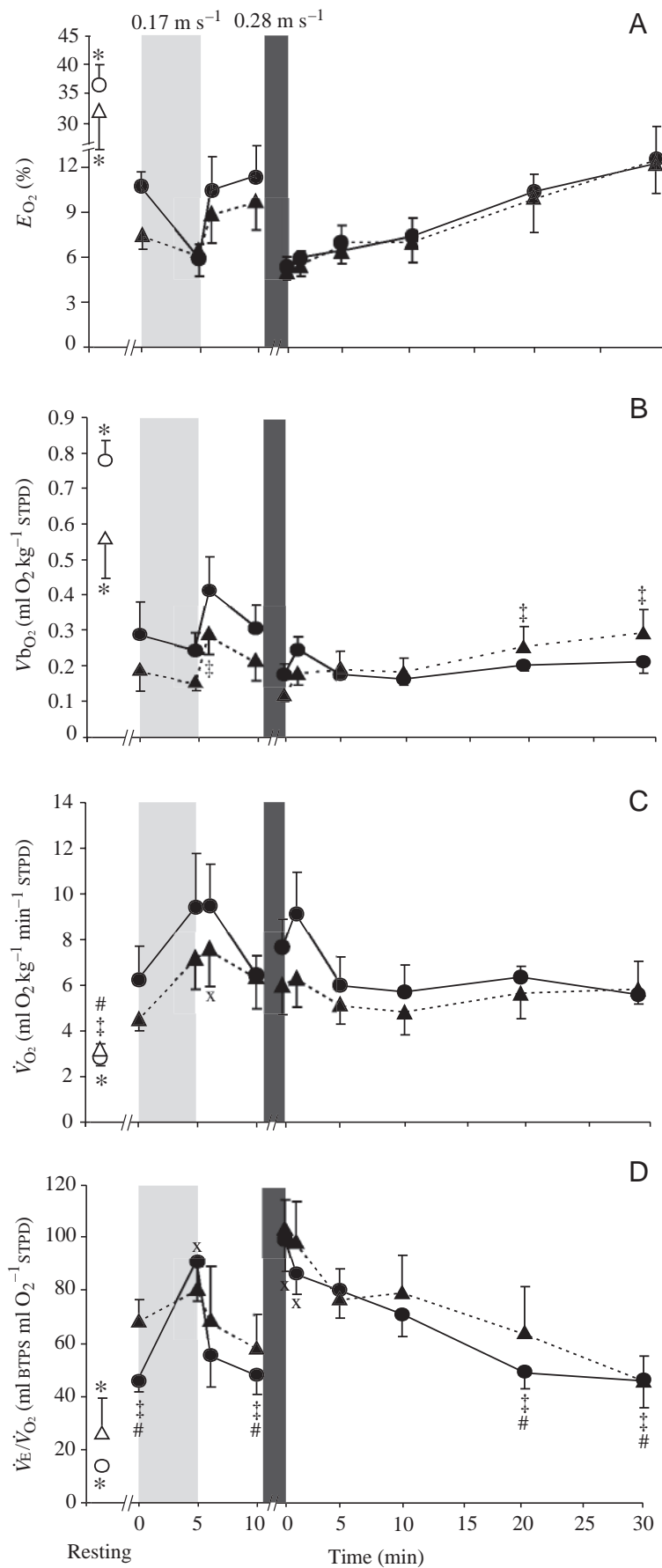


Fig. 2. Ventilation of septum removed (SR)- (triangles) and sham-operated (SO)-tegus (circles) resting during the night (open symbols) and at rest and activity during the day (filled symbols). The start of exercise at 0.17 m s^{-1} and the beginning of the recovery period after exercise at 0.28 m s^{-1} are defined as zero. Exercise at 0.17 m s^{-1} (grey bar) was terminated after 5 min, whereas exercise at 0.28 m s^{-1} (black bar) lasted till exhaustion of the animal. T_{TOT} , duration of total respiratory movements; f_R , breathing frequency; V_T , tidal volume; \dot{V}_E , total ventilation rate. Values are means \pm S.E.M., $N=5$. *Significantly different ($P \leq 0.05$) from all other values of a group; †significantly different ($P \leq 0.05$) between SO- and SR-tegus; ‡significantly different ($P \leq 0.05$) from pre-exercise; #significantly different ($P \leq 0.05$) from 0.17 m s^{-1} ; §significantly different ($P \leq 0.05$) from 0.28 m s^{-1} .



SR-tegus exercising at 0.17 m s⁻¹ compared with SO-animals (29.7 and 19.2 min, respectively) but at 0.28 m s⁻¹ endurance was greater in SO- than in SR-tegus (6.3 and 2.6 min, respectively; Fig. 4).

Discussion

In this study, tegus were forced to exercise on a motorised treadmill, which is not a natural situation for lizards. When we attempted to train tegus daily at 0.17 m s⁻¹ before experimentation, most of them would display defensive behaviours after a few days and they could not be motivated to run. Therefore, the tegus used in this study were not subject to training before experimentation. Additionally, tegus are highly active during the day and move frequently, especially at high temperatures. Therefore, not all tegus were resting quietly on the treadmill during the pre-exercise phase, even when left undisturbed for 30–60 min or when the treadmill was covered by a cloth, which was tested by way of trial before experimentation. Running was initiated when tegus started moving spontaneously, normally after 5 min of rest. The data thus obtained during the pre-exercise period may be elevated above the true resting condition of tegus during the day.

Tupinambis teguixin (earlier *T. nigropunctatus*; Avila-Pires, 1995) attain a maximum speed of 5.1 m s⁻¹ during quadrupedal and 4.3 m s⁻¹ during bipedal running (Urban, 1965). In general, however, lizards are only capable of maintaining such high velocities for short periods of time. Metabolism during these sprint events is largely anaerobic (Bennett, 1972, 1973; Bennett and Dawson, 1972; Bennett and Licht, 1972; Farmer and Hicks, 2000). *Iguana iguana*, for instance, reaches $\dot{V}_{O_{2max}}$ between 0.06 and 0.14 m s⁻¹ (Mitchell et al., 1981) and \dot{V}_{O_2} declines between 0.28 and 0.56 m s⁻¹ (Wang et al., 1997), which is only a small fraction (6.3%) of their maximum running speed. The greater speed for *T. merianae* in the present study (0.28 m s⁻¹) represents approximately 5% of their

Fig. 3. Ventilation of septum removed (SR)- (triangles) and sham-operated (SO)-tegus (circles) resting during the night (open symbols) and at rest and activity during the day (filled symbols). The start of exercise at 0.17 m s⁻¹ and the beginning of the recovery period after exercise at 0.28 m s⁻¹ are defined as zero. Exercise at 0.17 m s⁻¹ (grey bar) was terminated after 5 min, whereas exercise at 0.28 m s⁻¹ (black bar) lasted until exhaustion of the animal. E_{O_2} , oxygen extraction; $\dot{V}_{B_{O_2}}$, oxygen uptake per breath; \dot{V}_{O_2} , oxygen consumption; \dot{V}_E/\dot{V}_{O_2} , air convection requirement. Values are means \pm S.E.M., $N=5$. *Significantly different ($P \leq 0.05$) from all other values of a group; #significantly different ($P \leq 0.05$) from the pre-exercise; ‡significantly different ($P \leq 0.05$) from 0.17 m s⁻¹; †significantly different ($P \leq 0.05$) from 0.28 m s⁻¹. BTPS, body temperature, barometric pressure saturated; STPD, standard temperature and pressure, dry.

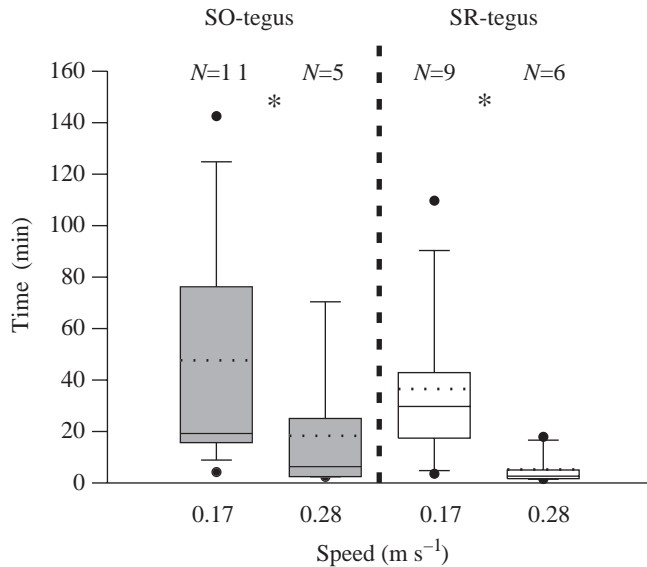


Fig. 4. Endurance of *Tupinambis merianae* with intact post-hepatic septum (PHS) (grey boxes) and with surgically removed PHS (white boxes) at 0.17 and 0.28 m s⁻¹. The lower boundary of a box indicates the 25th percentile, the solid line within the box marks the median, and the upper boundary of the box indicates the 75th percentile. Error bars above and below the box indicate the 90th and 10th percentiles. Means are presented as stippled lines and outliers as solid points. *Significant difference ($P \leq 0.05$) between 0.17 and 0.28 m s⁻¹ in a group of tegus.

maximum running velocity. To match the lower speed (0.17 m s⁻¹), tegus did not even need to walk continuously, but sometimes rested until they reached the end of the belt and then started to walk or run until they reached the other end of the box, when they again rested. To match the faster speed (i.e. 0.28 m s⁻¹), however, they had to walk continuously. This upper speed lies at the threshold at which \dot{V}_{O_2} reaches maximum and starts to decline with increasing velocity. The increased metabolic demands are subsequently covered by increasing anaerobic metabolism (Bennett, 1972, 1973; Bennett and Dawson, 1972; Bennett and Licht, 1972; Farmer and Hicks, 2000). The measured endurance indicates the importance of this speed range in tegus, as they are capable of sustaining 0.17 m s⁻¹ for approximately 20 min, even without PHS. Endurance time at 0.28 m s⁻¹ is greatly reduced in both groups and the high mean in SO-tigus is mainly based on one specimen, which showed a threefold greater endurance than the others. Therefore, the speed of 0.17 m s⁻¹ reflects the performance of tegus with respect to mainly aerobic activity, where a sustained efficiency of lung ventilation is necessary. At 0.28 m s⁻¹, on the other hand, \dot{V}_{O_2} still contributes greatly to the overall energy requirements, but anaerobic metabolism plays a more significant role.

Role of the post-hepatic septum

Tegus with intact PHS increased V_T during light exercise, whereas SR-tigus were not able to increase V_T while walking on the treadmill. In contrast, SR-tigus showed the highest V_T

directly after exercise. This implies that the PHS helps maintain a minimum space for lungs and that this volume can be increased by costal movements during low-speed treadmill locomotion. In tegus without PHS the viscera are displaced cranially (Klein et al., in press) especially during exercise, when the lateral undulation of the trunk induced by abdominal muscles causes greater pressures in the body cavity (Farmer and Hicks, 2000). According to Carrier (1987b), a mechanical constraint on the hypaxial muscles prevents lizards from breathing and running simultaneously. This limitation in ventilation, however, becomes more severe at greater speeds than the ones tested in this study and therefore \dot{V}_E may decrease in tegus running at greater velocities as a result of the speed dependent axial constraint. At 0.28 m s⁻¹, SO-tigus already showed a lower V_T than at 0.17 m s⁻¹, indicating that the axial constraint may play a significant role at higher velocities in running tegus. Due to the increase in f_R , however, \dot{V}_E also increased. During exercise, SR-tigus partially compensated for their generally low V_T by increasing f_R to a greater extent than in the SO-tigus, but still could not attain the \dot{V}_E of the latter group.

SR-tigus and SO-tigus showed nearly identical values for \dot{V}_E/\dot{V}_{O_2} . This was due to lower values for both \dot{V}_E and \dot{V}_{O_2} in SR-tigus compared with SO-animals. Despite a lack of significant differences in \dot{V}_E/\dot{V}_{O_2} between SR- and SO-tigus, the data suggest that without PHS, tegus are limited in their ability to respond to elevated metabolic demands. The SR-animals also needed longer to recover from exercise. The latter was indicated by the rise in $V_{B_{O_2}}$ after exercising at 0.28 m s⁻¹. Even 30 min after exercise, this value was greater than in the pre-exercise condition.

During resting conditions at night, $V_{B_{O_2}}$ tended to be lower ($P=0.0959$) in SR-tigus than in SO-tigus. The lack of the PHS possibly affected oxygen uptake negatively in tegus, but their endurance seemed to be unaffected. It is conceivable that chronic aerobic insufficiency increases the ability to sustain anaerobic activity in this group. This could be coupled with compensation through an increase in breathing frequency, an increase in pulmonary perfusion, or both. Furthermore, a reduction in lung volumes, which accompanies the removal of the PHS (Klein et al., 2003) could reduce the dead space of the lungs, while the volume and surface area of the gas exchange tissue remain unchanged.

Breathing pattern during rest and activity

The ventilatory pattern of *Tupinambis*, resting at night, consisted of regular breaths of long duration and constant frequency. The values for f_R and V_T obtained in this study ($f_R=3.6$ breaths min⁻¹; $V_T=10.5$ ml kg⁻¹) differ somewhat from those of Hlastala et al. (1985) for *T. teguixin* (earlier *T. nigropunctatus*; Avila-Pires, 1995; $f_R=8.21$ breaths min⁻¹; $V_T=9.6$ ml kg⁻¹) and Abe (1987) for *T. merianae* (earlier *T. teguixin*; Avila-Pires, 1995; $f_R=4.9$ breaths min⁻¹; $V_T=6.3$ ml kg⁻¹), as f_R in the former study is greater whereas in the latter V_T is less than in the present study. The differences may be due to differences in handling of the animals, to

different mask techniques or could represent species differences.

During exercise, breathing pattern was altered in SO- and SR-tegus and was characterised by breaths of high flow velocities and shorter duration. These findings are consistent with those of Carrier (1987a) and Wang et al. (1997). SO-tegus nearly doubled V_T at 0.17 m s^{-1} , and even while exercising at 0.28 m s^{-1} they were able to increase V_T . These results contrast with those for *V. exanthematicus* and *I. iguana* (Wang et al., 1997), where V_T showed no difference during exercise compared to pre-exercise values, but increased significantly during the recovery from speeds of 0.28 m s^{-1} and greater.

Gular pumping as additional breathing mechanism during locomotion was not observed in tegus as has been described for *Varanus* (Owerkowitz et al., 1999). Instead, the present results are consistent with the hypothesis that the PHS serves as a breathing aid during locomotion, and even after rupture of this structure the animals are not capable of evoking other mechanisms for supporting aerobic activity.

Tegus with intact PHS showed the greatest \dot{V}_{O_2} during exercise at 0.17 m s^{-1} and in the first minute of recovery from both speeds tested. The air convection requirement for oxygen, however, revealed a marked hyperventilation during both running events as well as at the beginning of the recovery period after exercising at 0.28 m s^{-1} . Following walking at 0.17 m s^{-1} , however, air convection requirements immediately returned to pre-exercise values. There is reason to believe that $\approx 0.17 \text{ m s}^{-1}$ is a reasonable approximation of the speed used by tegus when they are foraging (W. K., personal observation). At 0.28 m s^{-1} or faster, even the large observed increase in \dot{V}_E may not be sufficient to match the high aerobic demands, and thus the E_{O_2} falls. The increase in \dot{V}_{O_2} and \dot{V}_E directly after exercise, the slow return of \dot{V}_E/\dot{V}_{O_2} to pre-exercise values and the greatly reduced endurance are also consistent with this hypothesis. However, as flow rate was relatively low, the possibility of rebreathing exists, and would also be indicated by a low oxygen consumption and marked hypoventilation. More studies on the locomotor performance together with measurements of lactate production and lactate removal of tegus with and without PHS are needed to test the hypothesis that intact tegus recover faster from strenuous exercise.

Work of breathing

A change in minute ventilation can be produced in three different ways: (1) a change only in f_R , (2) a change only in V_T or (3) a combination of changes in f_R and V_T . Elastic work of breathing (\dot{W}) is defined as:

$$\dot{W} = (f_R / 2CL) \times V_T^2, \quad (1)$$

(modified after Crosfill and Widdicombe, 1961), where CL represents compliance of the lung. Therefore, the most economical breathing pattern consists of breaths at high frequencies and low tidal volume, where changes in \dot{V}_E are most economically produced solely by varying f_R (Milsom and Vitalis, 1984). The breathing pattern of resting tegus corresponds well with this hypothesis as V_T is kept within a

narrow range ($8\text{--}14 \text{ ml kg}^{-1}$) and a substantial increase in V_T is only shown during or immediately after exercise. On the other hand, f_R varied from $3.5 \text{ breaths min}^{-1}$ during rest at night to $25\text{--}30 \text{ breaths min}^{-1}$ resting during the day, and during exercise f_R increased even further ($40\text{--}50 \text{ breaths min}^{-1}$). This very high f_R value possibly represents the maximum breathing frequency in *Tupinambis*, as there is no pause between the breathing events and T_{TOT} is low. To match the greater metabolic demands during exercise, \dot{V}_E can only be increased by increasing V_T . Since the work of breathing increases with the square of V_T , the resulting breathing pattern is not energy efficient.

In conclusion, the PHS plays an important role in the breathing system in *Tupinambis*. It acts as a mechanical barrier to separate the lungs from the viscera, thereby increasing the efficiency of costal breathing. Especially during activity the PHS is essential for increasing \dot{V}_E by a combined increase in f_R and V_T , whereas tegus without a PHS rely solely on an increase in f_R to cope with the increasing metabolic demands during activity. Whether the PHS directly affects physiological parameters such as ventilation-perfusion ratio further remains to be elucidated.

List of symbols used

CL	lung compliance
f_R	breathing frequency
V_T	tidal volume
T_{TOT}	duration of total respiratory movements
$V_{B_{O_2}}$	oxygen uptake per breath
E_{O_2}	oxygen extraction coefficient
\dot{V}_{O_2}	rate of oxygen consumption
\dot{V}_E	total ventilation rate
\dot{W}	elastic work of breathing

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