

## Changes in pulmonary blood flow do not affect gas exchange during intermittent ventilation in resting turtles

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### SUMMARY

The breathing pattern of many different air-breathing vertebrates, including lungfish, anuran amphibians, turtles, crocodiles and snakes, is characterized by brief periods of lung ventilation interspersed among apnoeas of variable duration. These intermittent ventilatory cycles are associated with characteristic increases in pulmonary blood flow and tachycardia. In animals with central vascular shunts, the rise in pulmonary blood flow during ventilation is associated with the development of left-to-right (L–R) cardiac shunt (pulmonary recirculation of oxygenated blood returning from the lungs). By contrast, a large net right-to-left (R–L) shunt (pulmonary bypass) normally prevails during apnoea. The cardio-respiratory interaction and the changes in cardiac shunting have been suggested to improve pulmonary gas exchange but the benefits of L–R shunting on pulmonary gas transport have not been studied experimentally. The present study measured pulmonary gas exchange in fully recovered, freely diving turtles, where changes in pulmonary blood flow were prevented by partial occlusion of the pulmonary artery. Prevention of L–R shunt during ventilation did not impair CO<sub>2</sub> excretion and overall, oxygen uptake and CO<sub>2</sub> excretion did not correlate with changes in pulmonary blood flow. We conclude that increases in pulmonary blood flow associated with ventilation are not required to maintain resting rates of oxygen uptake and CO<sub>2</sub> excretion in resting animals.

Key words: reptile, turtle, periodic ventilation, pulmonary blood flow, cardiac shunt, left-to-right shunt, gas exchange, CO<sub>2</sub> excretion, respiratory gas exchange ratio.

### INTRODUCTION

Animals with an intermittent ventilatory pattern normally increase pulmonary blood flow ( $\dot{Q}_{\text{pul}}$ ) and heart rate ( $f_{\text{H}}$ ) during the brief periods of lung ventilation, providing a temporal matching of perfusion and ventilation of the lungs that may improve gas exchange efficacy (e.g. Johansen et al., 1970; Shelton, 1970; Shelton and Burggren, 1976; Lillywhite and Donald, 1989; Wang and Hicks, 1996) (cf. Hopkins et al., 1996). In animals with central vascular shunts, the rise in  $\dot{Q}_{\text{pul}}$  during ventilation is associated with the development of a left-to-right (L–R) cardiac shunt (pulmonary recirculation of oxygenated blood returning from the lungs) whereas a large net right-to-left (R–L) shunt (pulmonary bypass) predominates during apnoea. These hemodynamic changes are particularly evident in freshwater turtles (Shelton and Burggren, 1976; White et al., 1989; Wang and Hicks, 1996).

The physiological consequences of cardiac shunts on pulmonary gas exchange are not clear (Burggren, 1987; Hicks and Wang, 1996). R–L shunts lower arterial oxygen content because of the venous admixture and the resulting decrease in systemic oxygen delivery has been suggested to induce hypometabolism (Hicks and Wang, 1999; Platzack and Hicks, 2001). Such a reduction in oxygen requirement could be an important mechanism for extending aerobic dive durations but remains to be demonstrated in recovered and freely diving animals.

The effects of L–R shunt that returns oxygenated blood to the lungs are not easily predicted but functional consequences have been suggested (cf. Hicks and Wang, 1996). One hypothesis is that the increased oxygenation of pulmonary arterial blood caused by L–R shunt, facilitates pulmonary CO<sub>2</sub> excretion (Ackerman and White,

1979) (cf. White, 1985). According to this hypothesis, the L–R shunt increases pulmonary arterial hemoglobin oxygenation, which, in turn, elevates pulmonary arterial partial pressure of CO<sub>2</sub> ( $P_{\text{CO}_2}$ ) through the Haldane effect and, therefore, enlarges the  $P_{\text{CO}_2}$  gradient between the pulmonary capillaries and lung gas (White, 1985; White et al., 1989). However, a theoretical analysis of L–R shunting on CO<sub>2</sub> excretion provided conflicting results (Hicks and Wang, 1996). Thus, the change in pulmonary arterial  $P_{\text{CO}_2}$  is determined by the slope of the CO<sub>2</sub> dissociation curve, the magnitude of the Haldane effects, the degree of the L–R shunt as well as other physiological variables (Hicks and Wang, 1996) rendering *a priori* predictions concerning the role of L–R shunt on CO<sub>2</sub> excretion extremely difficult.

The objective of this study was to experimentally investigate the effects of changes in  $\dot{Q}_{\text{pul}}$  and net cardiac shunts on pulmonary gas exchange in an intermittently breathing vertebrate with cardiac shunts. For the present study, we chose the freshwater turtle, *Trachemys scripta* because this species normally exhibit intermittent breathing associated with large changes in cardiac shunt patterns (Shelton and Burggren, 1976; White, 1985; Wang and Hicks, 1996). In addition, the cardiovascular anatomy lends itself to a level of instrumentation and experimental manipulation not easily obtainable in other animals. This enabled us to manipulate  $\dot{Q}_{\text{pul}}$  in free diving turtles while measuring gas exchange and ventilation. By inflating a vascular occluder placed around the common pulmonary artery, we prevented the normal rise in  $\dot{Q}_{\text{pul}}$  and the associated L–R shunt during ventilation. Simultaneously, partially occluding pulmonary blood flow would result in a net R–L shunt during ventilation. If indeed L–R shunt facilitates CO<sub>2</sub> excretion, an abolishment of L–R

shunt during ventilation should result in CO<sub>2</sub> retention, which will be evident as a transient decrease in the respiratory gas exchange ratio. Conversely, if R–L shunt induces a hypometabolic state, then oxygen uptake should be reduced.

## MATERIALS AND METHODS

### Animals and instrumentation

Freshwater turtles (*Trachemys scripta* Gray) with a body mass ranging from 1.6 to 1.8 kg were purchased from Lemberger (Oshkosh, WI, USA) and freighted by air to the University of California at Irvine, USA. Here, the turtles were kept in open aquaria with free access to water and dry basking areas allowing for behavioral thermoregulation. They were fed fish but food was withheld for at least four days prior to surgery. For anesthesia, a short piece of soft rubber tubing was inserted through the glottis for artificial ventilation at 8–15 min<sup>-1</sup> with a tidal volume of 10–20 ml kg<sup>-1</sup> using a gas mixture consisting of 30% O<sub>2</sub>, 3% CO<sub>2</sub> (balance N<sub>2</sub>) prepared by a gas mixer (GF-3, Cameron Institute, TX, USA). This gas mixture passed through a Halothane vaporizer (Dräger, Lübeck, FRG, Germany) set at 4% to induce anesthesia. The Halothane level was reduced to 0.5–1% after 5–15 min when the turtles no longer responded to tactile stimulation. The heart and central blood vessels were exposed by removing a 4×5 cm portion of the plastron using a bone saw. The common pulmonary artery was freed from the systemic vessels in the aortic trunk and a vascular occluder (*In Vivo* Metric, Healdsburg, CA, USA) was placed around the common pulmonary artery. Short sections (1.5 cm) of the left pulmonary artery and the left aortic arch vessels were freed from the connecting tissue in order to place the two blood flow probes (2R, Transonic System, Ithaca, NY, USA). Acoustical gel (Mohawk Medical Supply, NY, USA) was injected around the probe to enhance the signal. Leads from the probes and the occluder were carried out ventrally and strapped to the carapace, and the excised piece of plastron was glued in place. Artificial ventilation was continued until the turtle regained spontaneous breathing.

### Measurement of blood flows, ventilation and gas exchange

Ventilation and gas exchange was determined in an experimental setup similar to that described by Glass et al. (Glass et al., 1983), where the turtle could move freely within a shielded holding tank (30×30×60 cm) covered by a grid forcing it to breathe at a funnel-shaped breathing hole with a diameter of 7 cm. The holding tank was covered on all sides to prevent visual disturbance. Airflow leaving the funnel covering the breathing hole was maintained constant at 500 ml min<sup>-1</sup> and continuously analysed using a Beckman OM-11 paramagnetic O<sub>2</sub> analyzer and a Beckman LB-2 CO<sub>2</sub> analyzer connected in series (Anaheim, CA, USA). Given the constant flow rate, oxygen consumption and CO<sub>2</sub> production could be calculated as the respective areas below or above the baseline values. In addition, this relationship was verified by artificially simulating exhalations with known gas compositions through the funnel. A pneumotachograph (0–5 LPM, Hans Rudolph, Shawnee, MO, USA) placed at the gas inlet and connected to a Validyne differential pressure transducer (DP45-14, Validyne Engineering Sales Corp., Northridge, CA, USA) recorded increases in gas flow during inhalations and decreases in gas flow during exhalations. The pneumotachograph was calibrated by manually simulating breaths of a known volume using a syringe, which was directly connected to the breathing funnel. As originally described by Funk et al. (Funk et al., 1986), we found that the integrated airflow signal of a given tidal volume decreased with increased frequency. Therefore, a calibration at several frequencies was necessary, and

the instantaneous breathing frequency (breath-to-breath frequency within a ventilatory period) of several turtles was analysed in detail to provide the exact correction factor. The flow probes were connected to a dual channel blood flow meter (T201, Transonic system) and  $f_{IH}$  was calculated on the basis of the instantaneous blood flow profiles. Total  $\dot{Q}_{pul}$  was calculated as  $2 \times \dot{Q}_{LPA}$  (left pulmonary artery) whereas the systemic cardiac output ( $\dot{Q}_{sys}$ ) was calculated as  $2.75 \times \dot{Q}_{LAo}$  (left aortic arch) (Wang and Hicks, 1996). All measurements were collected continuously at 15 Hz on a computer using Acknowledge data acquisition program (v. 3.0; Biopac System, Goleta, CA, USA).

### Experimental protocol

All animals recovered for a minimum of two days following surgery. On the morning of the measurements, the turtle was placed in the experimental setup and left undisturbed for 4–6 h. During this period, ventilatory and cardiovascular parameters approached the values previously reported in this species, under resting conditions. All physiological parameters were then collected for 60 min, and the vascular occluder was manually inflated to maintain  $\dot{Q}_{pul}$  at a level similar to that observed during apnoea prior to manipulation. The partial pulmonary artery occlusion was maintained for 60 min and then released. After this period, the turtle was left undisturbed for an additional 60 min. In two instances, pulmonary artery occlusion elicited noticeable activity and the measurements were aborted and repeated on the following day.

### Data analysis

Recordings of blood flows, ventilation and gas exchange were analyzed using Acknowledge (3.0) data analysis system (Biopac System). All experiments were divided into 18 ten-minute intervals (60 min control, 60 min of reduction of pulmonary blood flow and 60 min of subsequent control) and each interval was analysed for mean  $\dot{Q}_{pul}$ , mean  $\dot{Q}_{sys}$ , total ventilated volume, and total O<sub>2</sub> uptake and CO<sub>2</sub> excretion. For each 10 min interval, a mean and standard error of each of the five turtles were calculated. Because the analysis of ventilatory and cardiovascular parameters in turtles are complicated by the intermittent breathing pattern, a presentation of mean values, as described above, could mask possible effects of manipulating pulmonary blood flow on gas exchange. We, therefore, also present gas exchange values for each individual animal during the 10 min intervals as a function of ventilatory and cardiovascular variables.

### Statistics

The effects of partial occlusion of the physiological parameters were evaluated with a one-way analysis of variance (ANOVA) for repeated measures. In case of a significant effect, differences among means were assessed with a Student–Newman–Keuls test. To further evaluate the effects of  $\dot{Q}_{pul}$  on gas exchange, linear regression of data points was conducted. In all statistical analyses, a fiducial limit for significance of  $P < 0.05$  was applied. All data are presented as means  $\pm$  1 s.e.m.

## RESULTS

An example of blood flows and ventilation recorded during an experiment is shown in Fig. 1. Mean values for  $\dot{Q}_{pul}$  and  $\dot{Q}_{sys}$ , as well as minute ventilation ( $V_e$ ) and pulmonary gas exchange ratios (RE), are presented in Figs 2 and 3. The mean values obtained for ventilation, gas exchange and blood flows in the present study correspond well with previous reports on the same species at similar temperatures (e.g. Shelton and Burggren, 1976; Glass et al., 1983;

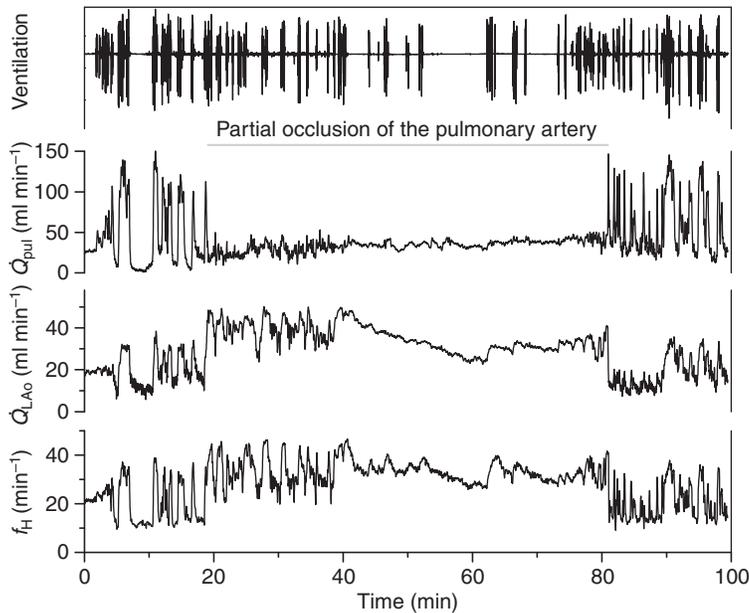


Fig. 1. An example of ventilation and blood flows in a turtle instrumented with flow probes on the left aortic arch (LAo) and the left pulmonary artery, as well as a vascular occluder around the common pulmonary artery. During the first 60 min, pulmonary blood flow ( $\dot{Q}_{pul}$ ) and heart rate ( $f_H$ ) increased substantially during ventilation, and caused the development of a net left-to-right (L-R) cardiac shunt pattern. This rise in  $\dot{Q}_{pul}$  was then prevented by partial occlusion of the common pulmonary by inflation of the vascular occluder for the following 60 min. As a consequence, a net cardiac right-to-left (R-L) shunt prevailed even during ventilation. Blood flows and ventilation are also shown during 60 min after releasing the occlusion.

Milsom and Chan, 1986; Wang and Hicks, 1996). Partial occlusion of the common pulmonary artery lowered  $\dot{Q}_{pul}$  to the levels measured during apnoea and elicited a small rise in  $\dot{Q}_{sys}$  (Figs 1 and 2). The manipulation of pulmonary artery blood flow abolished the net L-R cardiac shunt ( $\dot{Q}_{pul}/\dot{Q}_{sys} > 1$ ) during ventilation and induced a chronic R-L shunt throughout the ventilatory cycle, as reflected in a significant decrease of  $\dot{Q}_{pul}/\dot{Q}_{sys}$  to values below 1 (Fig. 2). This altered cardiac shunt pattern did not affect overall  $V_e$  or RE during the 60 min period of partial occlusion (Fig. 3). Upon releasing the occlusion, the normal hemodynamic changes associated with ventilation and apnoea were rapidly restored (see Fig. 1). In some individuals,  $\dot{Q}_{pul}$  increased drastically following release of the occlusion. A similar pattern of changes in blood flow and ventilation was observed in all experimental animals.

All individual data points for gas exchange over 10 min intervals for all five turtles are presented in Fig. 4. These results demonstrate that measured values of rate of oxygen uptake ( $\dot{V}_{O_2}$ ), rate of  $CO_2$  excretion ( $\dot{V}_{CO_2}$ ) and RE were correlated with  $V_e$  but were independent of total  $\dot{Q}_{pul}$  and  $\dot{Q}_{pul}/\dot{Q}_{sys}$ .

#### DISCUSSION

The turtles studied in the present study exhibited the large changes in  $\dot{Q}_{pul}$  and cardiac shunt patterns during ventilation that are characteristically reported for many air-breathing ectothermic vertebrates (White and Ross, 1966; White, 1968; Johansen et al., 1970; Shelton, 1970; Lillywhite and Donald, 1989). These hemodynamic changes associated with ventilation are particularly pronounced in turtles where  $\dot{Q}_{pul}$  and  $f_H$  increase several-fold during ventilation compared with apnoea (White and Ross, 1966; Shelton and Burggren, 1976; Wang and Hicks, 1996). The rise in  $\dot{Q}_{pul}$  during ventilation is primarily caused by lowered pulmonary vascular resistance as vagal tone on smooth muscle surrounding the pulmonary artery is reduced (Burggren, 1977; Milsom et al., 1977; Hicks, 1994; Wang et al., 2001). The intermittent ventilatory pattern causes systematic changes in pulmonary gas composition (Lenfant et al., 1970; Burggren and Shelton, 1979; Boutilier and Shelton, 1986; Hicks and White, 1992). Thus, as apnoea proceeds, oxygen removal from the lung vastly exceeds  $CO_2$  excretion and the RE consequently decreases (Burggren and Shelton, 1979). However, when ventilation resumes, RE increases

above the metabolic respiratory quotient (RQ) and may even exceed values greater than 2 (Burggren and Shelton, 1979; Glass and Johansen, 1979).

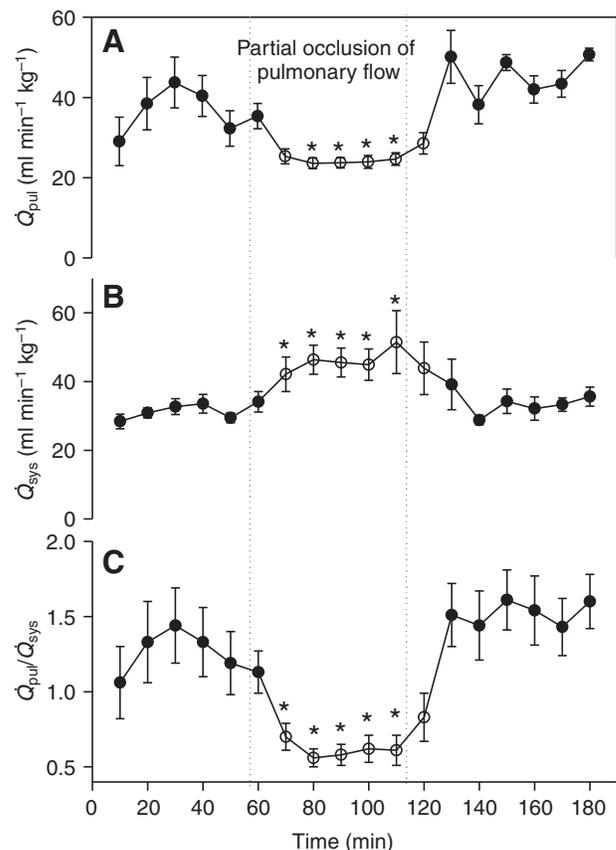


Fig. 2. Blood flows and cardiac shunt pattern before, during and after partial occlusion of the common pulmonary artery. (A) Mean pulmonary blood flow ( $\dot{Q}_{pul}$ ); (B) mean systemic blood flow ( $\dot{Q}_{sys}$ ); (C)  $\dot{Q}_{pul}/\dot{Q}_{sys}$ . Values are means  $\pm$  1 s.e.m. ( $N=5$ ) over 10 min periods. Values significantly different from control conditions are marked with an asterisk ( $P < 0.05$ ).

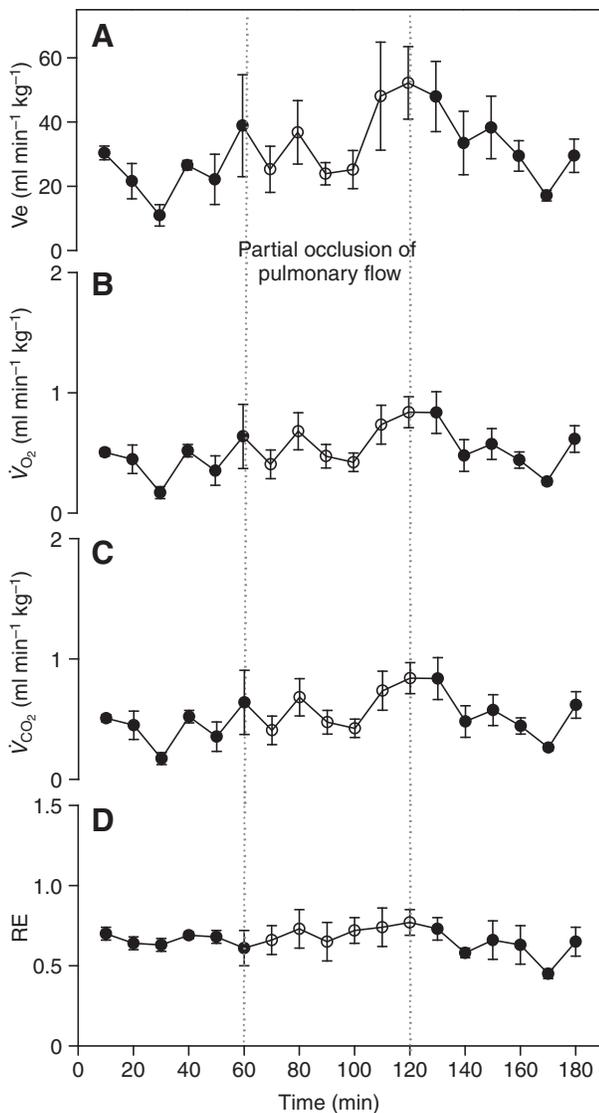


Fig. 3. Ventilation and gas exchange before, during and after partial occlusion of the common pulmonary artery. (A) Pulmonary ventilation ( $V_e$ ); (B) oxygen uptake ( $\dot{V}_{O_2}$ ); (C)  $CO_2$  excretion ( $\dot{V}_{CO_2}$ ) (D) respiratory gas exchange ratio (RE). Values are means  $\pm$  s.e.m. ( $N=5$ ) over 10 min period. Values significantly different from control conditions are marked with an asterisk ( $P<0.05$ ). No mean values during manipulation are significantly different than those obtained during the preceding control period.

A central aim of the present study was to investigate the hypothesis that net L–R cardiac shunt during the ventilatory periods augments the cyclic excretion of  $CO_2$  (Ackerman and White, 1979; White, 1985). L–R shunt increases hemoglobin oxygen saturation ( $HbO_{2sat}$ ) of pulmonary arterial blood relative to right atrial blood. This has been proposed to increase  $HbO_{2sat}$  of pulmonary arterial blood and lower blood  $CO_2$  affinity through the Haldane effect, which would increase  $P_{CO_2}$  in the pulmonary arterial blood. This elevated  $P_{CO_2}$  would then increase the  $P_{CO_2}$  gradient between pulmonary capillary blood and the lungs, and facilitate  $CO_2$  excretion into the lungs (White, 1985). The magnitude of this effect depends on the magnitude of the L–R shunt, the  $CO_2$  dissociation curve and oxygenation differences between the two atria but the complex interaction between these various physiological parameters renders theoretical predictions difficult (Hicks and Wang, 1996). However,

if L–R shunt contributes significantly to  $CO_2$  excretion, the experimental prevention of L–R cardiac shunt during ventilation by partial occlusion of the pulmonary artery should have led to a reduction in pulmonary  $CO_2$  excretion. This effect would best be evaluated as a reduction in the RE because this ratio would not be affected by altered metabolism. Our manipulation of  $\dot{Q}_{pul}$  successfully prevented L–R shunt during ventilation and maintained a net R–L shunt ( $\dot{Q}_{pul}/\dot{Q}_{sys}<1$ ) during both ventilatory periods and apnoea. The results of the present study demonstrate that eliminating net L–R shunt had no significant effect on pulmonary gas exchange in recovered animals and our study, therefore, does not support the hypothesis that L–R shunt during ventilation facilitates  $CO_2$  excretion in turtles at rest.

This study showed that overall gas exchange of resting turtles was not influenced by the reduction of  $\dot{Q}_{pul}$  and the induction of a chronic R–L shunt. A previous study investigating V/Q (ventilation-perfusion) distributions on the efficacy of gas exchange, indicated that a 10-fold change in  $\dot{Q}_{pul}$  and 6-fold change in  $\dot{Q}_{pul}/\dot{Q}_{sys}$  had no effect on overall  $\dot{V}_{CO_2}$  and  $\dot{V}_{O_2}$  in artificially ventilated, anesthetized turtles (Hopkins et al., 1996). Our study extends these observations to a fully recovered, freely diving turtle and underscores the notion that improved efficacy of lung function at high  $\dot{Q}_{pul}$  does not affect overall gas exchange of resting animal. Resting gas exchange is maintained both at high and low pulmonary blood flow, with R–L shunt ( $\dot{Q}_{pul}/\dot{Q}_{sys}<1$ ) or L–R shunt ( $\dot{Q}_{pul}/\dot{Q}_{sys}>1$ ). Consequently, we suggest that in animals with low resting oxygen demands, increases in  $\dot{Q}_{pul}$  associated with ventilation are not required to meet or maintain resting  $\dot{V}_{O_2}$  and  $\dot{V}_{CO_2}$ .

The observation that a prevailing R–L shunt ( $\dot{Q}_{pul}/\dot{Q}_{sys}<1$ ) did not reduce  $\dot{V}_{O_2}$  contrasts with previous studies on anesthetized and artificially ventilated turtles, where arterial hypoxemia, resulting either from reductions in inspired oxygen levels or a vagally induced R–L cardiac shunt, triggered a significant reduction in  $\dot{V}_{O_2}$  (Hicks and Wang, 1999; Platzack and Hicks, 2001). In these acute studies, the fully anesthetized turtles were artificially ventilated, and the 30–70% reductions in  $\dot{V}_{O_2}$  were fully reversed by injections of 2,4-dinitrophenol, which uncouples the mitochondria and shows that normal  $\dot{V}_{O_2}$  could be sustained at the reduced level of systemic oxygen delivery ( $\dot{Q}_{sys} \times$  arterial  $O_2$  concentration) (Hicks and Wang, 1999; Platzack and Hicks, 2001). Such hypoxemic-induced hypometabolism would be a powerful mechanism for extending aerobic dive times in animals with central vascular shunts (Hicks and Wang, 2004). In the present study, the absence of a significant reduction in  $\dot{V}_{O_2}$  with low  $\dot{Q}_{pul}$  and  $\dot{Q}_{pul}/\dot{Q}_{sys}<1$  suggests that hypometabolism may not occur in recovered, freely diving turtles. In previous studies on anesthetized *Trachemys*, systemic oxygen delivery was reduced by over 50% through reductions in inspired oxygen levels and by over 60% during electrical stimulation of the right vagal afferent nerves (Hicks and Wang, 1999; Platzack and Hicks, 2001). In freely diving turtles, it is possible that the overall reduction in systemic oxygen delivery may not have been of sufficient magnitude to trigger a hypometabolic state. Unfortunately, we did not measure arterial blood gases, so although a net R–L shunt occurred ( $\dot{Q}_{pul}/\dot{Q}_{sys}<1$ ), the absolute reduction in systemic oxygen delivery could not be assessed.

Our overall findings do not necessarily negate the relative importance of altering  $\dot{Q}_{pul}$  and cardiac shunt patterns during intermittent ventilation. Whereas an increased  $\dot{Q}_{pul}$  alone did not affect resting pulmonary gas exchange, a low  $\dot{Q}_{pul}/\dot{Q}_{sys}$  lowers arterial oxygen content through the addition of venous admixture to arterial blood. Conversely, an elevation of  $\dot{Q}_{pul}$  during ventilation

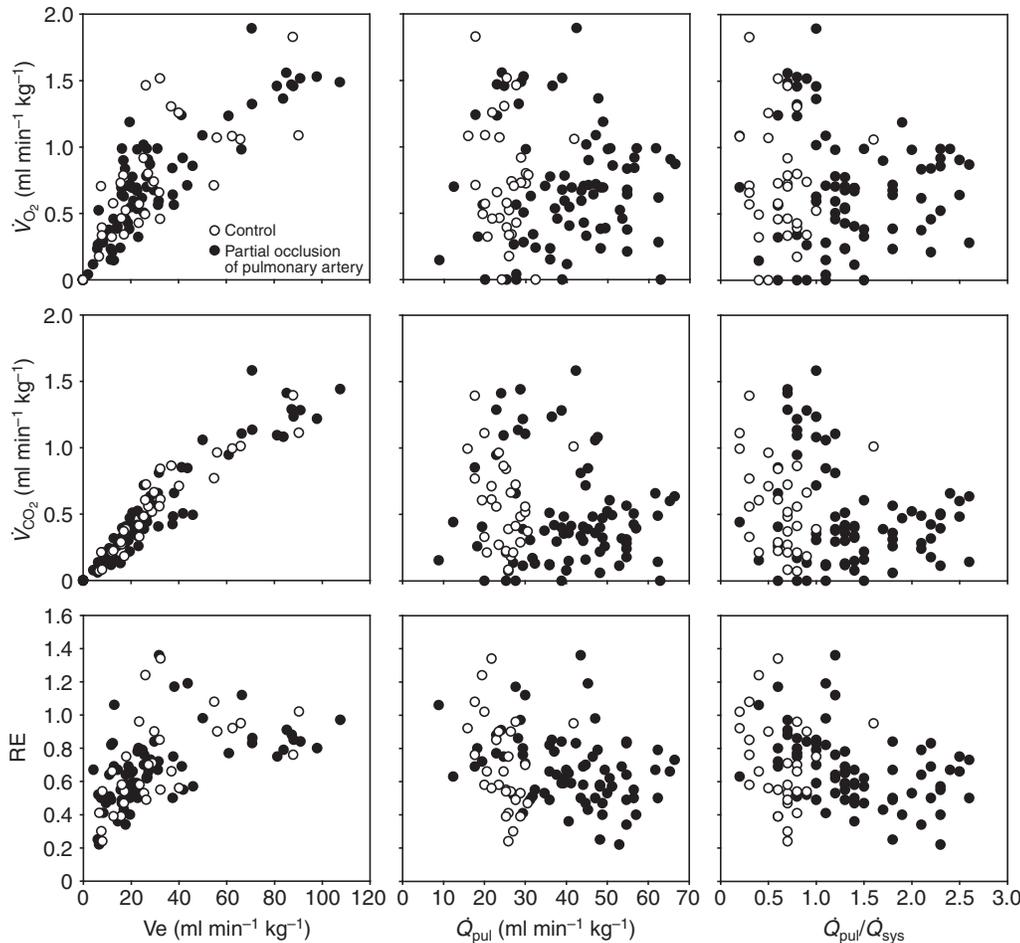


Fig. 4. Oxygen uptake ( $\dot{V}_{O_2}$ ),  $CO_2$  excretion ( $\dot{V}_{CO_2}$ ) and the respiratory gas exchange ratio (RE) as a function of minute ventilation ( $V_e$ , left panel), pulmonary blood flow ( $\dot{Q}_{pul}$ , middle panel) and  $\dot{Q}_{pul}/\dot{Q}_{sys}$  (right panel). Open circles represent periods of partial occlusion of the pulmonary artery, and closed circles represent pre- and post-occlusion. Each symbol represents the mean value determined every 10 min, during a three hour experimental protocol.

and the attending increases in  $\dot{Q}_{pul}/\dot{Q}_{sys}$  will elevate arterial blood oxygen content. If  $\dot{Q}_{pul}/\dot{Q}_{sys}$  were to remain relatively low during ventilation, as we demonstrated in this study, the resting oxygen demands will be satisfied but the result is accomplished under conditions of lowered arterial oxygen saturation, due to R-L shunt ( $\dot{Q}_{pul}/\dot{Q}_{sys} < 1$ ). Such a condition may, over the long term, influence the potential for aerobic diving.

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