

Short communication

Respiration and gas exchange during recovery from exercise in the American alligator

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Accepted 26 January 2000

Abstract

Low respiratory exchange ratios (R) occur in reptiles. In crocodylians they have been attributed to the loss of respiratory CO₂ into the urine and to the immediate exercise-history of an animal. In this study, expired ventilation (\dot{V}_E), oxygen consumption (\dot{V}_{O_2}), and carbon dioxide excretion (\dot{V}_{CO_2}) were measured during recovery from treadmill-exercise in the American alligator. Both \dot{V}_{O_2} and \dot{V}_{CO_2} decreased exponentially during recovery, but the decrease of \dot{V}_{CO_2} was greater than that of \dot{V}_{O_2} . By 55 min into recovery R fell to 0.3 ± 0.02 (mean \pm S.E.M). These low values of R may be partially attributable to hyperventilation that occurred during exercise. These data suggest that both hyperventilation during exercise and a metabolic acidosis deplete blood bicarbonate stores, contributing to a low R during recovery. We propose that the right to left cardiac shunt could facilitate restoration of these alkaline reserves and blood pH. © 2000 Published by Elsevier Science B.V. All rights reserved.

Keywords: Acid–base; Exercise; Gas exchange, respiratory exchange ratio; Reptiles, American alligator

1. Introduction

The respiratory exchange ratio (R) is the ratio of CO₂ production/O₂ consumption (also known as the respiratory quotient, RQ) and yields information about the type of food being catabolized

by an animal when in a steady state (West, 1979). Values range from 0.7 for fat catabolism to 1.0 for carbohydrate catabolism. However, in reptiles R ranges as low as 0.3 (Potter and Glass, 1931; Bennett and Dawson, 1976). Explanations for these values in crocodylians include a loss of respiratory CO₂ to the urine (Coulson and Hernandez, 1959, 1964; Grigg, 1978), experimental error, and a time lag in changes in alkaline reserve of the blood (reviewed in Grigg, 1978). Loss of respira-

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tory CO_2 to urine appears not to explain the low values found in crocodylians (Grigg, 1978; Coulson and Hernandez, 1983). Experimental error also seems an unlikely explanation. Hence, a time lag in alkaline changes in the blood is probably a source of the variability of R.

If an animal increases ventilation such that alveolar (or faveolar) P_{CO_2} declines below normal resting values (hyperventilation), it may take some time for alveolar P_{CO_2} and P_{O_2} to regain steady state because of CO_2 and O_2 stores in the body (West, 1979). Because CO_2 is stored in plasma and in the interstitial fluids as bicarbonate, it takes much longer for CO_2 than for O_2 to reach equilibrium (West, 1979). Initially with the onset of hyperventilation, R in the expired gas is elevated as the CO_2 stores are washed out of the body; the opposite occurs with the onset of hypoventilation (West, 1979). Low Rs reported for alligators have been associated with recovery from struggling (Hicks and White, 1992). More recently, we observed severe hyperventilation during treadmill-locomotion in alligators which could contribute to a time lag in alkaline reserves of the blood during recovery from exercise (Farmer and Carrier, 2000). Although we do not understand the reason for this hyperventilation, it is known that crocodylians have an unusually large pulmonary air-to-blood barrier thickness (Perry, 1993). It is possible that hyperventilation is necessary to maintain arterial saturation. We undertook this study to examine changes in respiratory and gas exchange parameters during recovery from exercise to determine how R varied.

2. Materials and methods

2.1. Animals

Five captive raised American alligators (*Alligator mississippiensis*) were kept in aquaria with basking platforms and both heat and full spectrum light sources. They experienced a photoperiod of 14:10 h light:dark. They were fed a diet of goldfish, smelts, mice, rats, and eggs. The animals' weight ranged from 1.18 to 1.66 kg; the mean weight was 1.34 kg.

2.2. Training

Animals were trained to walk on a treadmill over the course of several months. By exposing them to short training sessions (several min of walking with 10 min intervals for rest) two to three times a week, the endurance of the animals improved until they could sustain repeated bouts of 4 min of continuous walking interspersed with 25 min of rest.

2.3. Ventilation and gas exchange

To measure ventilation and gas exchange, a mask was constructed out of the tip of a 30 ml plastic syringe. Two ports were drilled into the syringe and flexible, Hytrel[®] tubing (Hans Rudolph, Inc., Kansas City, MO) with an inside diameter of 9 mm was glued to the ports. Flow control units (Ametek, Pittsburgh, PA) were used to pull fresh air ($\text{FI}_{\text{O}_2} = 0.2093$ and $\text{FI}_{\text{CO}_2} = 0.0003$) through the mask at an approximate rate of 5.4 L/min. This was the biased flow determined to eliminate rebreathing by the alligators during their most vigorous exercise. A pneumotach (Hans Rudolph, Inc., Kansas City, MO) was placed in the line upstream from the mask; a portion of the gas flowing through the mask was diverted and pulled through Drierite (anhydrous calcium sulfate) and then through oxygen (Beckman OM-11, Fullerton, CA) and carbon dioxide (Ametek CD-3A, Pittsburgh, PA) analyzers. The mask was sealed with epoxy over the nares. The mouth of the animal was sealed with duct tape to ensure all respiratory gases were collected. The ventilation and gas exchange system was calibrated by injecting known volumes of gas into the mask before it was attached to the animal.

2.4. Data collection and analysis

Signals from the gas analyzers and the differential pressure transducer of the pneumotach were converted to digital form using an AD converter (Biopac System, Goleta, CA) and stored on a Macintosh computer. Signals were sampled at a rate of 50 Hz and analyzed with Acqknowledge software (Biopac System Goleta, CA).

Respiration is reported at body temperature, ambient pressure, and saturated with water vapor (BTPS). Gas exchange is expressed at standard temperature and pressure, dry (STPD) (West, 1979). Values are means and standard error about the mean (mean \pm S.E.M.). Pre-exercise values for expired ventilation (\dot{V}_E), tidal volumes (\dot{V}_T), respiratory frequency (f_R), oxygen consumption (\dot{V}_{O_2}), and carbon dioxide excretion (\dot{V}_{CO_2}) were obtained several hours after the animal had been instrumented. Exercise values of \dot{V}_{O_2} , \dot{V}_{CO_2} , \dot{V}_E , f_R , and \dot{V}_T were collected during the last min of a 4-min exercise bout at 0.44 m/sec. Recovery values are an average of one min of data for the following periods: 1, 5, and 15 min after cessation of exercise. Three min of data were averaged for: 25–28 min and 55–58 min of recovery. It was necessary to increase the period examined during the latter part of recovery due to a low respiratory frequency. These values were used to compute respiratory gas exchange ratios ($\dot{V}_{CO_2}/\dot{V}_{O_2}$); air convection requirements (\dot{V}_E/\dot{V}_{O_2} and \dot{V}_E/\dot{V}_{CO_2}); extraction ($\dot{V}_{O_2}/\dot{V}_E \cdot FI_{O_2}$); as well as to estimate alveolar P_{CO_2} with the alveolar gas equation assuming a dead space volume of 4.2 ml/kg (Hicks and White, 1992). Paired *t*-tests were used to determine statistically significant ($P < 0.05$) differences.

2.5. Experimental protocol

All animals had fasted for at least 4 days before the experiments. They were brought from the animal care facility to the experimental chamber the night before data were collected. The experimental chamber was kept at 30°C.

Our protocol was determined by two factors. First, as part of a previous study, we were interested in collecting data of gas exchange and ventilation during exercise on alligators in a steady state (Farmer and Carrier, 2000). The focus of this study was to determine whether or not alligators experienced a mechanical constraint on simultaneous running and breathing. To assess this, we allowed an animal several hours on the treadmill after instrumenting to calm down from the handling and masking before pre-exercise data were collected. After monitoring pre-exercise, the treadmill was started and the animal exercised for 4 min. After

no less than 25 min of rest, an exercise trial was repeated at a higher speed. This procedure was repeated until the animals had walked at 0.17, 0.31, and 0.44 m/sec, which was the highest speed for which we were able to get sustained locomotion. Hence we were able to collect 1 min of data after the walking alligators had reached steady state, which occurred within 1.5–2 min after the start of exercise. At the end of the exercise period at the highest speed studied, the animals were left undisturbed on the treadmill while recovery was monitored for 1 h.

The second reason for this protocol is that recovery from an intense, anaerobic burst of struggling has been studied previously (Coulson and Hernandez, 1964, 1980, 1983). We were interested in recovery from exercise in which the animal was unencumbered from breathing freely, either during or after the exercise period. Hence we avoided intense exercise where excessive handling could suppress ventilation. We also avoided exercise in water for the same reason. Because we wanted the animals to work at a pace that was sustainable for at least 4 min but we wanted the most intense exercise possible within these parameters, we used repeated bouts of exercise at progressively higher speeds.

3. Results

During exercise all animals hyperventilated severely (Fig. 1). Expired ventilation declined immediately during the min following exercise by 73%. This was due to a large decrease in respiratory frequency and a smaller drop in tidal volume (Fig. 1). By 25 min of the recovery period, ventilation had decreased to pre-exercise values. The equation that best describes the ventilation during and after exercise is given in Table 1.

Table 1
Equations fit to expired ventilation and gas exchange during and after treadmill-locomotion

Equation	r ²
$\dot{V}_E = -248.48 \log(t) + 414.8$	0.988
$\dot{V}_{CO_2} = 11.615 \times 10^{(-0.027t)}$	0.951
$\dot{V}_{O_2} = 8.469 \times 10^{(-0.017t)}$	0.869

Oxygen consumption and carbon dioxide excretion declined upon the cessation of exercise (Fig. 1), however the rate of decrease was faster for \dot{V}_{CO_2} than for \dot{V}_{O_2} . This point is illustrated by the

equations that describe the rates of decline (Table 1). \dot{V}_{O_2} during the first minute of recovery was not significantly different from exercise ($P < 0.05$). \dot{V}_{O_2} was significantly different from exercise by

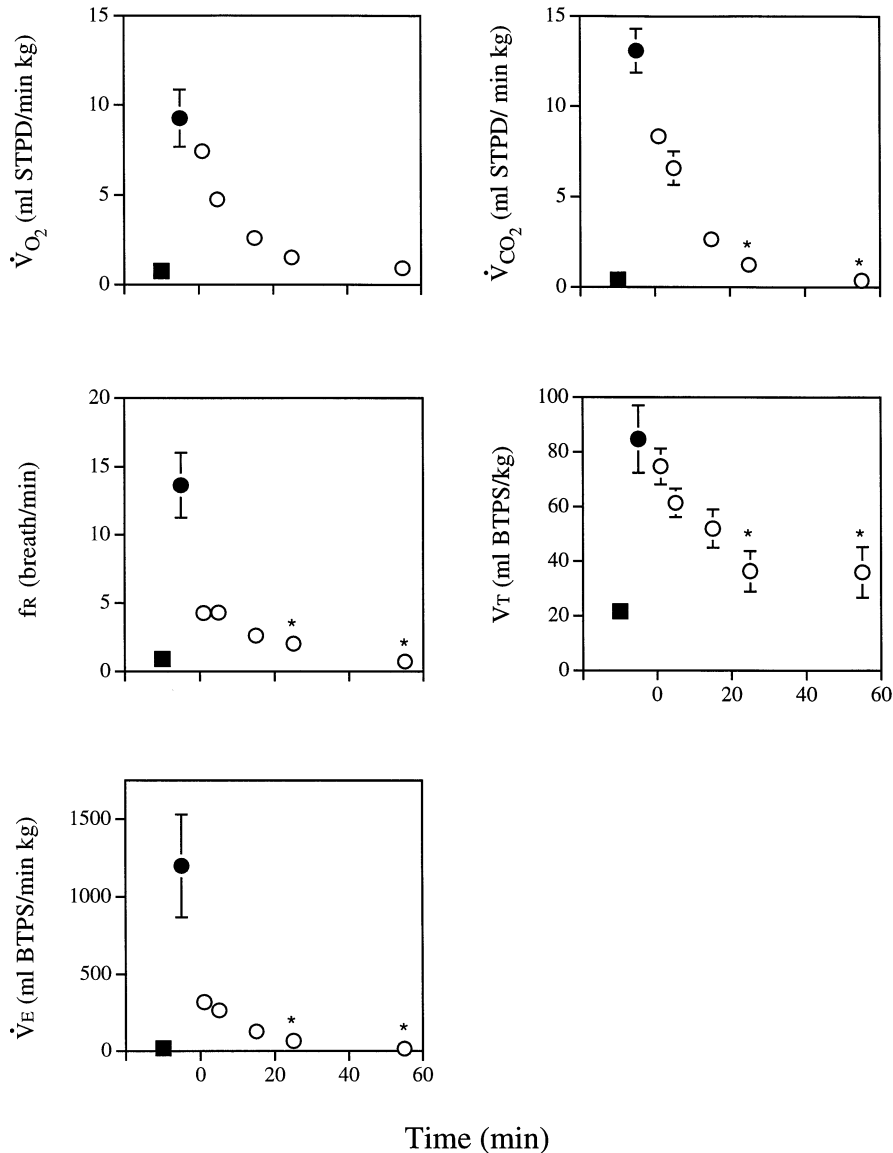


Fig. 1. Mean ($n = 5$) and standard errors for oxygen consumption (\dot{V}_{O_2}), carbon dioxide excretion (\dot{V}_{CO_2}), expired ventilation (\dot{V}_E), tidal volume (V_T), and respiratory frequency (fR) before exercise, during the last minute of exercise, and during recovery. Recovery begins at $t = 0$. If error bars are not seen it is because they are contained within the symbols. Symbols are as follows: solid square = pre-exercise, solid circle = exercise, open circle = recovery, * indicates a value that is not statistically different from pre-exercise (i.e. $P > 0.05$ paired t -test).

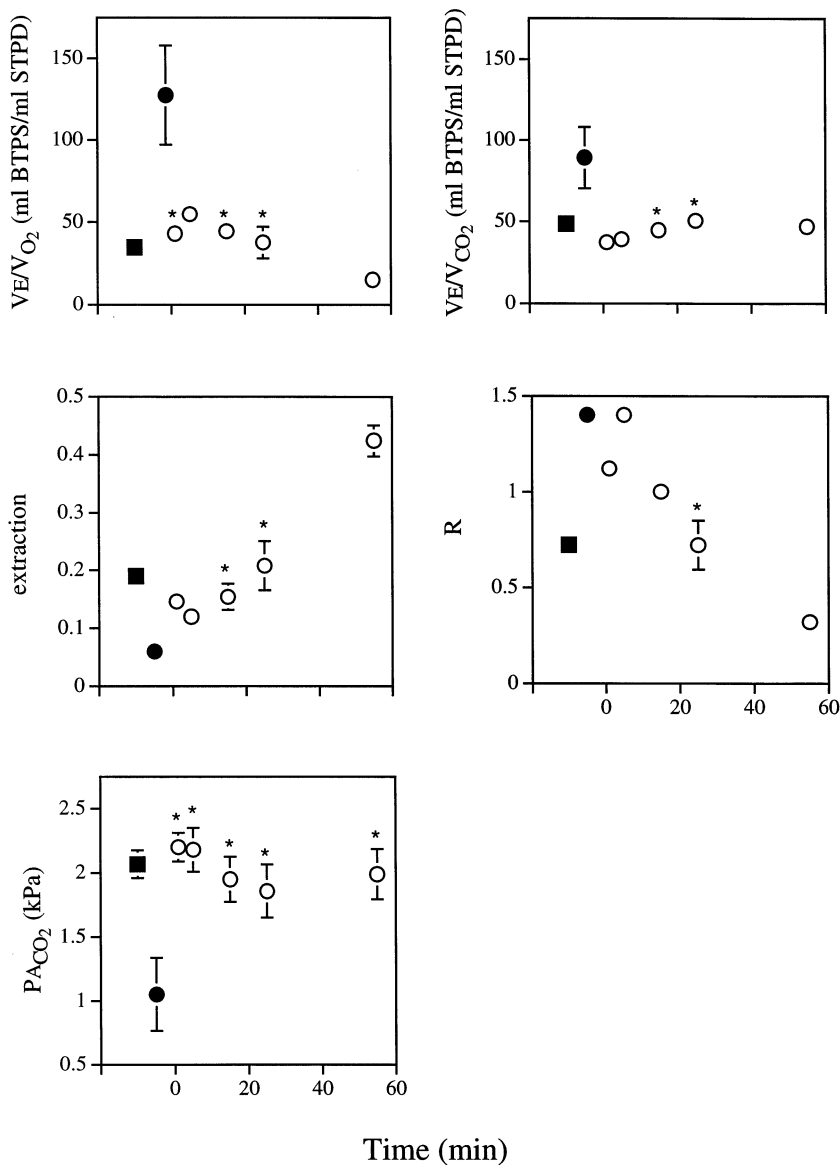


Fig. 2. Mean ($n = 5$) and standard errors for air convection requirements (\dot{V}_E/\dot{V}_{O_2} and \dot{V}_E/\dot{V}_{CO_2}), extraction, respiratory exchange ratio (R), and estimates of lung P_{CO_2} (P_{ACO_2}) before exercise, during the last minute of exercise, and during recovery. Recovery begins at $t = 0$. If error bars are not seen it is because they are contained within the symbols. Symbols are as follows: solid square, pre-exercise; solid circle, exercise; open circle, recovery, * indicates a value that is not statistically different from pre-exercise (i.e. $P > 0.05$ paired t -test).

min five ($P < 0.05$). In contrast, \dot{V}_{CO_2} was significantly different from the exercise value during the first minute of recovery ($P < 0.05$).

Respiratory gas exchange ratios declined significantly ($P < 0.05$) from exercise levels during the

first minute of recovery. Then they rebounded to exercise levels by 5 min into recovery, after which they continued to decline. However, they remained above pre-exercise values until 25 min (Fig. 2). They were significantly lower than pre-

exercise values by 55 min of recovery, reaching 0.3 ± 0.02 (mean \pm S.E.M) (Fig. 2).

The changes in air convection requirements with cessation of exercise were different for O_2 than for CO_2 . \dot{V}_E/\dot{V}_{CO_2} declined immediately during the first min of recovery to a mean value that was significantly less than pre-exercise and remained so throughout 5 min of recovery ($P < 0.05$). In contrast, although there was a large decline in \dot{V}_E/\dot{V}_{O_2} during the first minute of recovery, \dot{V}_E/\dot{V}_{O_2} did not drop below pre-exercise levels. Air convection requirements for oxygen were not significantly different from pre-exercise values for the first, fifteenth, and twenty-fifth minute of recovery (Fig. 2). At 5 and 55 min and of recovery, \dot{V}_E/\dot{V}_{O_2} was significantly less ($P < 0.05$) than during pre-exercise (Fig. 2).

During recovery, O_2 extraction immediately increased from exercise values and remained elevated during the 60 min period (Fig. 2) By 15 min of recovery extraction was not significantly different from pre-exercise values ($P > 0.05$). By 55 min of recovery extraction had increased to approximately 40%, significantly elevated above pre-exercise levels ($P < 0.05$).

Estimates of lung P_{CO_2} obtained with the alveolar gas equation immediately increased with the cessation of exercise (Fig. 2). The values were not significantly different from pre-exercise values throughout the recovery period (Fig. 2).

4. Discussion

Our analysis of ventilation and respiratory gas exchange during and after exercise indicates highly variable gas exchange ratios. Under the conditions of this study, R varied from a normal range (between 0.7 and 1.0) before exercise, to 1.4 during exercise and at 5 min after exercise, to 0.3 by 55 min of recovery. We suspect that both hyperventilation and a metabolic acidosis contribute to this pattern of gas exchange. R can be greatly elevated simply by hyperventilation and both the ventilatory equivalents and the estimate of lung P_{CO_2} indicate a severe hyperventilation occurred during exercise. However, if an elevated R were caused by hyperventilation alone, a rapid

return to pre-exercise levels, or below pre-exercise levels, is expected when the hyperventilation ceases. Although R significantly declined for the first minute of recovery, it did not drop below 1 in spite of the return to pre-exercise lung P_{CO_2} . We suggest this elevation in R , that remained until 25 min of recovery, is due to a metabolic acid load incurred during exercise. Hence, repeated bouts of treadmill-exercise that were sustainable for 4 min in alligators appear to cause both a metabolic acid load and a respiratory alkalosis. Both of these factors can deplete blood bicarbonate stores. It has long been known that one way to restore blood bicarbonate is to hypoventilate so that CO_2 is retained. However unlike mammals, crocodylians have another way to restore blood bicarbonate and pH. They can shunt blood around the lungs (a right to left shunt).

The right to left shunt has long been suggested to be important for diving ectothermic tetrapods in facilitating oxygen uptake from the lungs as the dive progresses (White, 1978, 1985; Grigg, 1992). By sequestering CO_2 in the tissues and away from the lungs, a right to left shunt may aid the continued uptake of pulmonary oxygen in spite of a declining Alveolar-arterial O_2 gradient by decreasing the rate at which P_{50} in the lung increases (White, 1978; Grigg, 1992). Furthermore, a build up of CO_2 in the tissues would facilitate oxygen unloading there, due to the Bohr effect. Previous studies have found mixed results regarding whether or not the right to left shunt occurs during forced diving (White, 1969; Jones and Shelton, 1993).

Recovery from treadmill-exercise is not analogous to forced diving because the alligators were free to breathe whenever they desired thereby replenishing lung O_2 stores. However, as with diving animals, a right to left shunt would divert CO_2 rich blood away from the lung and might facilitate the retention of CO_2 during recovery. This could help reestablish blood bicarbonate buffer stores and restore blood pH, while still enabling oxygen uptake in the lungs. Such a blood flow pattern may partially explain the extremely low respiratory gas exchange value (0.3) and the large extraction (40%) found at 55 min of recovery. Hence these respiratory data raise some

provocative questions regarding potential cardiovascular patterns that may be present during and after exercise. Because anesthesia and catheterization can induce hyperventilation and alter blood gas and bicarbonate parameters (Forster and Pan, 1988), we undertook this study to characterize the respiratory parameters in animals that have not undergone these procedures. However, preliminary data indicate that alligators develop a right to left shunt after treadmill-exercise (C.G. Farmer, L. Hartzler, and J.W. Hicks, unpublished).

Acknowledgements

We are indebted to J.W. Hicks for many insights, for helpful comments on the manuscript, and for the use of equipment. We are grateful to J.W. Hicks, K. Packard, G. Packard, and to the Florida Freshwater Fish and Game Commission for supplying us with alligators. We thank Jon Tlachac for his assistance with training the animals. This study was funded by NIH # 1F32-HL09796-01 to C.G. Farmer and NSF IBN-9807534 to D.R. Carrier.

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