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SHORT COMMUNICATION

There is no limitation for CO_2 excretion across the lung in exercising American alligators (*Alligator mississippiensis*)

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ABSTRACT

Vertebrates utilize various respiratory organs such as gills, lungs and skin in combination with diverse cardiovascular structures, including single-, three- and four-chambered hearts, to enable oxygen delivery and carbon dioxide removal. They also exhibit differences in aerobic and anaerobic metabolism during exertion, but the cardiorespiratory gas transport of all vertebrates is a four-step process governed by Fick's Principle and Fick's Law of Diffusion over the entire range of metabolic rates. Hillman et al. (2013) suggested that previous exercise studies have focused too narrowly on mammals and proposed that the cardiorespiratory system's excess capacity serves an evolutionary role in enhancing CO_2 excretion in non-mammalian vertebrates. In contrast, an analysis by Hicks and Wang (2021) concluded that vertebrates maintain effective gas exchange even at peak activity, finding no evidence of arterial hypercapnia at maximal oxygen consumption and thus challenging the proposal of significant limitations to pulmonary or branchial CO_2 efflux. In the present study, we investigated the limits for CO_2 exchange in exercising American alligators (*Alligator mississippiensis*) and provide evidence that the cardiorespiratory system is adequately built to sustain CO_2 excretion during strenuous exercise and maintain arterial P_{CO_2}, with no evidence of diffusion limitation for pulmonary CO_2 excretion.

KEY WORDS: Cardiovascular, Reptile, Crocodilian, Ectotherm, Activity, Blood gases

INTRODUCTION

Vertebrates rely on a wide range of respiratory organs, such as gills, lungs and skin, and a variety of cardiovascular structures, including single-chambered, three-chambered and four-chambered hearts, to deliver oxygen to the tissue and to remove carbon dioxide (Wang et al., 2019). Significant differences also exist in their reliance on aerobic and anaerobic metabolism during physical exertion, as evidenced by net lactate accumulation (Hedrick et al., 2015). Despite these anatomical and metabolic variations, the cardiorespiratory transport of O_2 and CO_2 follows a uniform four-step process involving convection and diffusion. Each step is defined quantitatively by fundamental mass transport equations: the convective phases (ventilation and cardiac output) adhere to Fick's Principle, while Fick's Law governs the diffusive phases.

In 1926, A. V. Hill concluded that oxygen transport reaches its maximum (the maximal rate of oxygen consumption, $\dot{V}_{O_2,max}$) during physical exertion and remains constant as a result of the limitations of the circulatory and respiratory systems (Mitchell et al., 1958). Consequently, any anatomical or functional feature that impairs O_2 transfer or $\dot{V}_{O_2,max}$ will limit the ability of animals to sustain their metabolic demands aerobically. During the past century, however, the cardiorespiratory limitations for $\dot{V}_{O_2,max}$ during maximal locomotor activity have been debated. The classical view holds that $\dot{V}_{O_2,max}$, in healthy normoxic subjects, is limited by cardiac output and muscle blood flow (e.g. Saltin and Calbet, 2006). This view is not universally accepted, as the integration of convective and diffusive transport reveals that both contribute to overall limitations (Wang and Hicks, 2002; Wagner, 2006).

In 2013, Hillman and colleagues conducted a comparative study of O_2 and CO_2 exchange and proposed a new hypothesis regarding the evolution of the vertebrate oxygen transport system (Hillman et al., 2013). They critiqued the oxygen-centric analysis leading to the concept of symmorphosis (Taylor and Weibel, 1981) for its limited phylogenetic scope and failure to account for the apparent excess capacity of the respiratory system. Hillman et al.'s analysis concluded that the cardiovascular system limits $\dot{V}_{O_2,max}$, while the respiratory system limits maximal CO_2 efflux in air-breathing ectotherms.

Ectothermic vertebrates can have a large anaerobic scope, and the resulting lactate efflux during maximal activity titrates blood bicarbonate stores, producing maximal $\dot{V}_{CO_2}$ that approaches a level 1.5–2 times higher than $\dot{V}_{O_2,max}$ (Hartzler et al., 2006). Hillman et al. (2013) proposed that natural selection enhances the respiratory system's ability to eliminate CO_2 in ectotherms, leading to a respiratory system that appears overbuilt when only considering O_2 transport. A subsequent review by Hedrick et al. (2015) hypothesized, 'If the respiratory system limits CO_2 efflux during $\dot{V}_{O_2,max}$, we would expect arterial hypercapnia because CO_2 is not completely eliminated at the respiratory surface, and the accumulation of CO_2 would result in arterial acidemia' (Hedrick et al., 2015) (see Fig. 1).

In a recent review, Hicks and Wang (2021) also compared gas exchange across the vertebrate spectrum. In contrast to the previous reviews (Hillman et al., 2013; Hedrick et al., 2015), it was concluded that even though vertebrates exhibit diverse respiratory organs and cardiac structures, sufficient O_2 and CO_2 exchange are maintained even at peak metabolic rates (Hicks and Wang, 2021). Additionally, arterial P_{O_2} remained high in most species at $\dot{V}_{O_2,max}$, and arterial P_{CO_2} was either unchanged or lower than at rest. These patterns in blood gases indicate that gas exchange structures pose no significant limitation for O_2 uptake nor CO_2 excretion during physical activity. A significant arterial hypercapnia at or near $\dot{V}_{O_2,max}$ is uncommon amongst exercising vertebrates.

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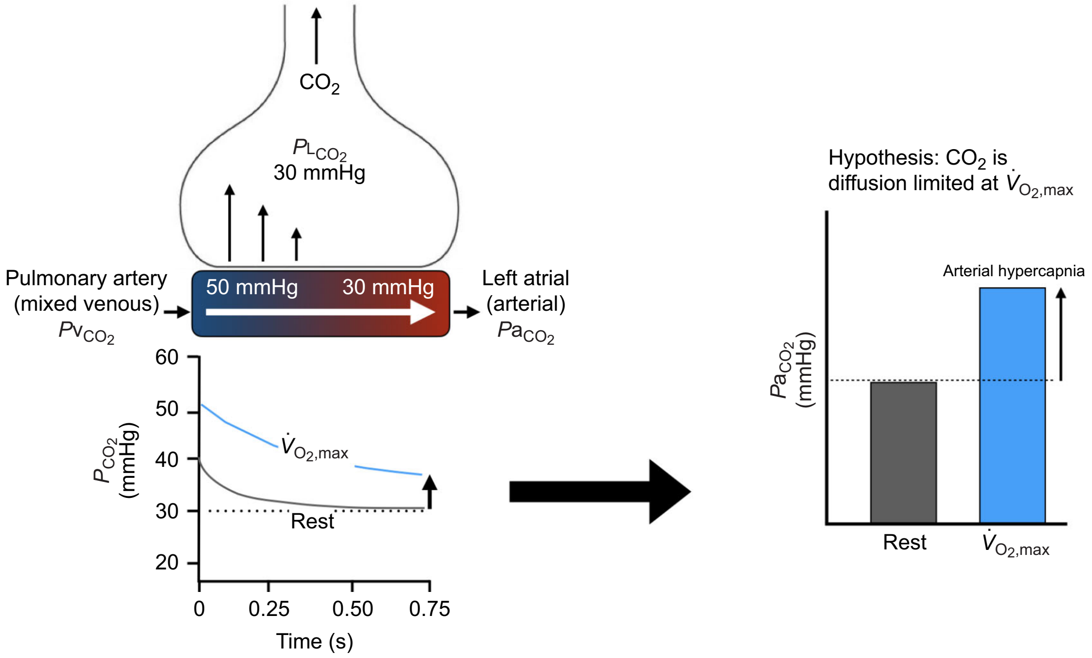

Fig. 1. A simple graphical model, showing incomplete equilibration of CO_2 between the lung gas and blood phase, as blood transverses the lung. If the respiratory system limits CO_2 efflux at $\dot{V}_{O_2,max}$, then CO_2 in the arterial blood (Pa_{CO_2}) would be greater than that in the lung gas (P_{LCO_2}) (left panel, upward arrow from Rest to $\dot{V}_{O_2,max}$). This would result in the accumulation of CO_2 in arterial blood, i.e. arterial hypercapnia (right panel), where Pa_{CO_2} would increase as hypothesized by Hedrick et al. (2015). Gas exchange graphical model modified from Scheid and Piiper (1980).

Given the discrepancies between different reviews and the inherent issues of variations in study design, our goal was to directly test the possible limits to CO_2 exchange in maximally active American alligators (*Alligator mississippiensis*).

MATERIALS AND METHODS

Experimental animals

Eleven 3-year-old American alligators, *Alligator mississippiensis* (Daudin 1801) (mass 2214±207 g), were used in the study. All animals were maintained in 378 and 567 l plastic containers with free access to water at temperatures between 24 and 28°C. They were fed commercial alligator food (Crocodilian Diet, Mazuri Exotic Animal Nutrition, St Louis, MO, USA) 3 times weekly and maintained on a 12 h:12 h light:dark cycle. The experiments were approved by the University of North Texas animal ethics committee (IACUC #17-001).

Surgical procedures

All animals were fasted for at least 10 days before instrumentation. On the day of surgery, animals were isolated, and a plastic bag containing cotton gauze saturated with isoflurane (Isothesia, Henry Schein Animal Health, Dublin, OH, USA) was held over the head of the animal. Once the righting reflex subsided, the alligator was weighed and placed on a stainless-steel surgical table in supine position. The glottis was intubated with soft Tygon® tubing for mechanical ventilation (Harvard Apparatus 665 ventilator, Harvard Apparatus, Holliston, MA, USA) at 7–10 breaths min^{-1} and a tidal volume of 25 ml kg^{-1}. Air for ventilation was pulled through an isoflurane vaporizer (FluTec vaporizer, FluTec, Ohmeda, OH, USA) set to make a mixture of 2% isoflurane with 98% room air. Once the animal reached a surgical plane of anesthesia, as determined by the lack of a pedal reflex, the isoflurane level was reduced to 1–1.5%.

The ventral skin was cleaned with Betadine solution and rinsed with 75% ethanol. Subcutaneous injections of a general antibiotic (0.1 ml kg^{-1}, 2.7% Baytril, Bayer Healthcare LLC, Shawnee Mission, KS, USA) and a general analgesic (1.1 mg kg^{-1} FluMeglumine, Flunixin Meglumine, Clipper Distributing Company, St Joseph, MO, USA) were then administered in the right rear limb. Lidocaine (4.5 mg kg^{-1} Lidoject, Henry Schein Animal Health) was injected sub-dermally above the sternum and an incision was made through the skin extending from the posterior edge of the pectoral girdle to the base of the sternum. The sternum was then partially split at the midline with a scalpel blade (size 22) to access the pericardium and the major arteries.

The left pulmonary artery was isolated by blunt dissection. A silk suture (4-0) was then passed under the vessel to briefly interrupt flow by retracting the vessel with a hemostat. The remnant of the ductus arteriosus was isolated to insert a PE 10 catheter (polyethylene tubing, Braintree Scientific, Braintree, MA, USA) with a heat-flared tip that was forwarded to a position at the junction with the pulmonary artery. The catheter was then secured in place with two 6-0 silk sutures and connected to PE 50 tubing containing heparinized saline (50 IU ml^{-1} in 0.9% NaCl). A short sleeve of PE tubing (PTFE 0.15, Braintree Scientific) was then slipped over the joint and treated with cyanoacrylic glue gel (Loctite, Henkel Corporation, OH, USA) and a bonding accelerant (Zip Kicker, Pacer Technology, ON, Canada). After catheterization, the left atrium was exposed by making a cut (0.5–1 cm) in the pericardium. The wall of the atrium was gently retracted using micro-hemostats, and small hole (<0.5 cm) was then cut to allow a PE 50 catheter with a bubble at the tip filled with heparinized saline (0.9% NaCl) to be inserted into the atrium. Left atrium tissue was fastened around the catheter with two 4-0 sutures and the pericardium was closed with 5-0 silk. Once in place, the catheters were tunneled under the skin and externalized through a dorsal perforation. The sternum and overlying skin were closed with a silk suture (2-0) and catheters were secured to the back of the animal.

At completion of the procedure, the animals were allowed to recover in plastic containers (50×40×40 cm) for 24 h in an environmental control room at 30°C on a 12 h:12 h light:dark cycle.

Physical activity

Control blood samples were drawn from both catheters and used to measure the blood parameters outlined below. Animals were then removed from the recovery container and exercised to exhaustion as previously described (Hartzler et al., 2006; Bagatto et al., 2012). Briefly, animals were placed ventral side up and allowed to flip over, righting themselves to ventral side down. This manipulation was repeated until the animals could no longer flip over. At this point, a second blood sample was immediately taken from both catheters. Catheters were heat-sealed, and the animals were returned to their holding containers. The activity study was conducted in a temperature-controlled room at 30°C. Upon completion of measurements, all animals were euthanized by a vascular injection of sodium pentobarbital (150 mg kg^{-1}) and tissues and organs extracted for additional studies.

Measurement of blood gases

Blood samples (500 µl) were drawn simultaneously from the two catheters into heparinized 1 ml syringes. Then, 20 µl of blood from each syringe was used to measure whole blood glucose and lactate concentrations using a glucose/lactate analyzer (2300 STAT Plus, YSI, Yellow Springs, OH, USA). Two 50 µl microhematocrit tubes were filled with blood from the two syringes and placed in a centrifuge (Micro-Hematocrit Damon/IEC Division) at 14,000 rpm for 5 min to measure hematocrit. The remaining blood in both syringes was used to measure blood P_{CO_2}, P_{O_2} and pH using a Radiometer BMS MK 2 Blood Micro-System (Radiometer, Copenhagen, Denmark) with the electrodes kept at 30°C using a circulating water bath (RM 6 RMA, LAUDA-Brinkmann LP, Delran, NJ, USA). Plasma bicarbonate values were calculated as described by Busk et al. (2000). Blood gases were measured before and after the activity protocol.

Statistical analysis

The effects of activity on blood parameters were analyzed with a repeated measures ANOVA (Statistica v13, StatSoft, Tulsa, OK, USA) with pre- and post-activity values used as the repeated factor and the source of the blood (left atrium or pulmonary artery) used as independent factors. A Neuman–Keuls *post hoc* test was used for pairwise comparisons of blood sample source (left atrium or pulmonary artery) as well as pre- and post-activity values. Data are presented as means±s.e.m., with statistical significance designated when $P \leq 0.05$.

RESULTS AND DISCUSSION

The time to exhaustion was 304±24 s and the number of flipping events required to reach exhaustion was 115±14. Time to exhaustion is similar to a previous study in American alligators (Hartzler et al., 2006). We did not measure oxygen consumption ($\dot{V}_{O_2}$) or carbon dioxide production ($\dot{V}_{CO_2}$), but a similar exercise protocol for exhaustive activity elicited a 10-fold elevation of $\dot{V}_{O_2}$ and a 28-fold rise in $\dot{V}_{CO_2}$ in alligators (Hartzler et al., 2006). The disproportionate rise in $\dot{V}_{CO_2}$ results from titration of tissue and plasma HCO_3^- by lactic acidosis and leads to a respiratory exchange ratio ($\dot{V}_{CO_2}/\dot{V}_{O_2}$) approaching 2.5 (Hartzler et al., 2006). Indeed, whole-blood lactate measured in mixed venous and arterial blood increased over 20-fold following exhaustive exercise (Fig. 2E) and the activity-induced lactic acidosis resulted in significant reductions in both mixed venous and arterial pH (Fig. 2C) as well as a 3-fold reduction in mixed venous and arterial [HCO_3^-] (Fig. 2D).

For clarity, measurements from the pulmonary artery are referred to as mixed venous, and samples from the left atrium are referred to as arterial. In the current study, the mixed venous P_{O_2} decreased significantly during activity, consistent with increased oxygen extraction during muscular activity (Fig. 2A). Interestingly, an activity-induced arterial hypoxemia was measured with a 15 mmHg reduction in arterial P_{O_2} (Pa_{O_2}) at the end of activity. A decrease in Pa_{O_2} could result from changes in *V*/*Q* distributions in the lung, intrapulmonary shunts (Powell and Gray, 1989; Wang et al., 1998) or a R–L shunt (Hicks, 2002). However, several lines of evidence support the concept that the respiratory system is near its functional limits for O_2 exchange when approaching maximum metabolic rates. A previous study (Hartzler et al., 2006) on American alligators using a similar exercise protocol reported a 28-fold increase in minute ventilation (V_E) following vigorous activity (from 30 to 525 ml kg min^{-1}). Using measures of Pa_{CO_2} in the current study and the gas exchange ratio (*R*) 2.25 after exhaustive exercise (Hartzler, et al., 2006), a large difference between lung and arterial P_{O_2} arises. This is similar to a widening of the alveolar–arterial (A–a) gradient for oxygen in the mammalian lung, and we predict that the A–aO_2 gradient would increase from less than 5 mmHg at rest to more 50 mmHg in exhaustive activity. The widening of the A–aO_2 gradient during activity is consistent with theoretical analysis suggesting the reptilian respiratory system is operating near the functional limit in terms of oxygen uptake (Wang and Hicks, 2004).

Mixed venous P_{CO_2} increased almost 2-fold during activity (Fig. 2B). Although the CO_2 load to the lung more than doubled, arterial P_{CO_2} (Pa_{CO_2}) remained unchanged from the control value (Fig. 2B). Hematocrit (Hct) in mixed venous and arterial blood was not affected by exhaustive activity (Fig. 2F). This is consistent with a recent review (see Hicks and Wang, 2021) showing in reptiles that at maximal metabolic demand, Pa_{CO_2} remains near or below resting values. Thus, there is no indication of any physiologically relevant diffusion limitations for CO_2.

Our measurements of mixed venous and Pa_{CO_2} demonstrate that the substantial increase in CO_2 load to the lung during exhaustive exercise did not result in arterial hypercapnia. This result does not support the hypothesis proposed by Hedrick et al. (2015) that the lung is diffusion limited for CO_2, but we argue that significant CO_2 retention would not occur at $\dot{V}_{O_2,max}$ because of the high diffusivity of CO_2. A recent review indicates that in reptiles, birds and most mammals, hyperventilation during exercise at or near $\dot{V}_{O_2,max}$ results in Pa_{CO_2} remaining at resting values or declining below resting Pa_{CO_2} (Hicks and Wang, 2021). There are exceptions, including some amphibians and, most notably, Thoroughbred racehorses (Hicks and Wang, 2021).

Our results do not directly refute the evolutionary hypothesis by Hillman et al. (2013), i.e. natural selection enhanced maximal CO_2 excretion in response to large CO_2 loads and thus resulted in a seemingly ‘over-built’ respiratory system. However, it is not clear that an over-built respiratory system is a general feature of air-breathing vertebrates. The ‘overbuilt’ concept originates from several lines of evidence, primarily from the mammalian and human literature. First, analysis of the oxygen transport system in mammals, within the framework of optimal design, gave rise to symmorphosis (Taylor and Weibel, 1981), dictating that all steps of the oxygen transport system have well-matched capacities to meet the organism’s functional demands. Analyses of various mammals

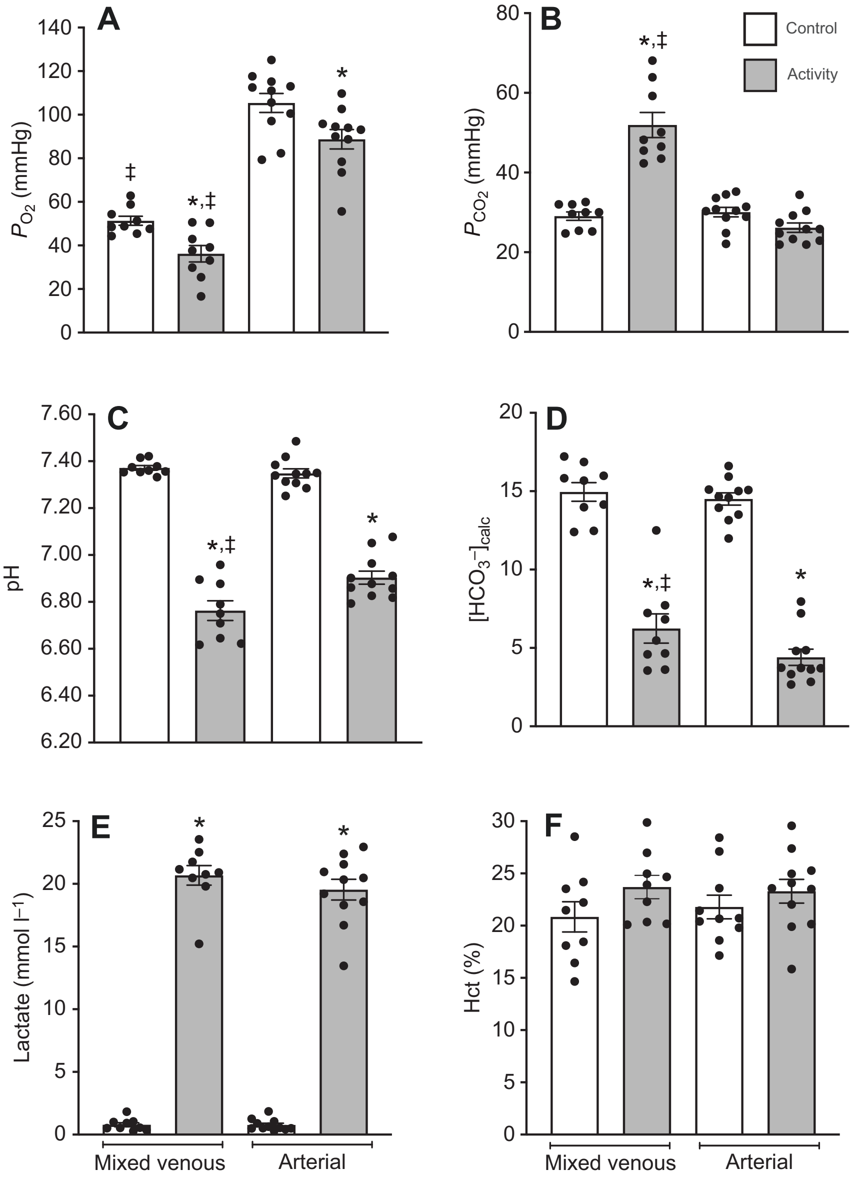

Fig. 2. Blood parameters measured in mixed venous and arterial blood of juvenile American alligators at rest (control) and after completion of a non-sustainable activity bout. (A) Partial pressure of oxygen (P_{O_2}), (B) partial pressure of CO$_2$ (P_{CO_2}), (C) pH, (D) calculated bicarbonate ($[HCO_3^-]_{calc}$), (E) whole-blood lactate and (F) hematocrit (Hct) in samples from the left pulmonary artery (mixed venous; *n*=9) and left atrium (arterial; *n*=11). Asterisks indicate a difference between baseline values and those after completion of the activity bout within either the arterial or the mixed venous blood based on the repeated measures ANOVA. Double daggers indicate a difference between the two sampling sites before or after the activity based on the repeated measures ANOVA.

within this context supports the notion that the respiratory system has excess capacity. The concept of symmorphosis has not been applied quantitatively to birds, reptiles or amphibians, and it is not clear how the idea apply across differences in metabolic strategy, variations in lung structure and respiratory mechanics and cardiovascular anatomies.

Second, the improvement in $\dot{V}_{O_2,max}$ with endurance exercise training reflects the upregulation of capacities at each step of the oxygen transport system, yet the human respiratory system remains virtually unchanged (Dempsey et al., 2020). Endurance athletes with elite $\dot{V}_{O_2,max}$ levels may or may not have enhanced diffusion capacities or lung volumes (Dempsey et al., 2020).

Evidence supporting the apparent 'overbuilt' nature of the mammalian respiratory system cannot, *a priori*, be extrapolated to all airbreathing vertebrates. The exercise training response in humans with improvements in $\dot{V}_{O_2,max}$ without major changes in the diffusion capacities and lung volumes may not universally apply across the vertebrate spectrum to endurance, particularly in ectotherms. The African clawed frog, *Xenopus laevis*, improved locomotor performance during training, yet liver glycogen concentration, heart mass and muscle citrate synthase activity were unaffected (Miller and Camillere, 1981). Studies in non-avian reptiles have routinely indicated the absence of a mammalian-like training effect (Gleeson, 1979; Garland et al., 1987; Conley et al., 1995; Thompson and Cowan, 1997). However, recent evidence suggests that green anoles improve locomotor performance after training (Husak et al., 2015). In varanid lizards, chronic electric stimulation of skeletal muscle increases mitochondrial volume density (Schaeffer et al., 2007) but treadmill exercise training had little effect on $\dot{V}_{O_2,max}$ (Szucsik et al., 2004). Finally, 16 weeks of treadmill training augmented $\dot{V}_{O_2,max}$ by 28% and doubled locomotor endurance in saltwater crocodiles (Owerkowicz and Baudinette, 2008). It remains unclear whether endurance training is widespread in ectothermic air breathers.

In conclusion, the hypothesis that arterial hypercapnia occurs during vigorous activity is not supported. In addition, there does not appear to be strong evidence that an 'overbuilt' respiratory system can be generally applied to all airbreathing vertebrates. A recent review of the literature (Hicks and Wang, 2021) and the results from this study indicate that the respiratory system of ectothermic reptiles is adequately 'built' to handle CO_2 exchange from rest to $\dot{V}_{O_2,\mathrm{max}}$.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: J.L.C., R.E., D.A.C., T.W., J.W.H.; Methodology: J.L.C., D.A.C., T.W., J.W.H.; Validation: D.A.C.; Formal analysis: J.L.C., D.A.C.; Investigation: D.A.C., T.W., J.W.H.; Resources: R.E., D.A.C.; Data curation: D.A.C.; Writing - original draft: D.A.C., T.W., J.W.H.; Writing - review & editing: J.L.C., R.E., D.A.C., T.W., J.W.H.; Supervision: D.A.C., J.W.H.; Project administration: D.A.C.; Funding acquisition: D.A.C.

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Data availability

All data will be provided upon reasonable request.

References

Bagatto, B., Crossley, D. A., Altimiras, J., Elsey, R. M. and Hicks, J. W. (2012). Physiological variability in yearling alligators: clutch differences at rest and during activity. *Comp. Biochem. Physiol.* **162**, 44-50. doi:10.1016/j.cbpa.2012.02.005

Busk, M., Overgaard, J., Hicks, J. W., Bennett, A. F. and Wang, T. (2000). Effects of feeding on arterial blood gases in the American alligator *Alligator mississippiensis*. *J. Exp. Biol.* **203**, 3117-3124. doi:10.1242/jeb.203.20.3117

Conley, K. E., Christian, K. A., Hoppeler, H. and Weibel, E. R. (1995). Heart mitochondrial properties and aerobic capacity are similarly related in a mammal and a reptile. *J.Exp. Biol.* **198**, 739-746. doi:10.1242/jeb.198.3.739

Dempsey, J. A., La Gerche, A. and Hull, J. H. (2020). Is the healthy respiratory system built just right, overbuilt, or underbuilt to meet the demands imposed by exercise? *J. Appl. Physiol.* **129**, 1235-1256. doi:10.1152/japplphysiol.00444.2020

Garland, T., Jr, Else, P. L., Hulbert, A. J. and Tap, P. (1987). Effects of endurance training and captivity on activity metabolism of lizards. *Am. J. Physiol.* **252**, R450-R456. doi:10.1152/ajpcell.1987.252.4.C450

Gleeson, T. T. (1979). The effects of training and captivity on the metabolic capacity of the lizard *Sceloporus occidentalis*. *J. Comp. Physiol.* **129**, 123-128. doi:10.1007/BF00798176

Hartzler, L. K., Munns, S. L., Bennett, A. F. and Hicks, J. W. (2006). Recovery from an activity-induced metabolic acidosis in the American alligator, *Alligator mississippiensis*. *Comp. Biochem. Physiol.* **143A**, 368-374. doi:10.1016/j.cbpa.2005.12.024

Hedrick, M. S., Hancock, T. V. and Hillman, S. S. (2015). Metabolism at the max: how vertebrate organisms respond to physical activity. *Compr. Physiol.* **5**, 1677-1703. doi:10.1002/cphy.c130032

Hicks, J. W. (2002). The physiological and evolutionary significance of cardiovascular shunting patterns in reptiles. *News Physiol. Sci.* **17**, 241-245. doi:10.1152/nips.01397.2002

Hicks, J. W. and Wang, T. (2021). Arterial blood gases during maximum metabolic demands: patterns across the vertebrate spectrum. *Comp. Biochem. Physiol. A* **254**, 110888. doi:10.1016/j.cbpa.2020.110888

Hillman, S. S., Hancock, T. V. and Hedrick, M. S. (2013). A comparative meta-analysis of maximal aerobic metabolism of vertebrates: implications for respiratory and cardiovascular limits to gas exchange. *J. Comp. Physiol.* **183B**, 167-179.

Husak, J. F., Keith, A. R. and Wittry, B. N. (2015). Making Olympic lizards: the effects of specialised exercise training on performance. *J. Exp. Biol.* **218**, 899-906. doi:10.1242/jeb.114975

Miller, K. and Camilliere, J. J. (1981). Physical training improves swimming performance of the African clawed frog *Xenopus laevis*. *Herpetologica* **37**, 1-10.

Mitchell, J. H., Sproule, B. J. and Chapman, C. B. (1958). The physiological meaning of the maximal oxygen intake test. *J. Clin. Invest.* **37**, 538-547. doi:10.1172/JCI103636

Owerkowicz, T. and Baudinette, R. V. (2008). Exercise training enhances aerobic capacity in juvenile estuarine crocodiles (*Crocodylus porosus*). *Comp. Biochem. Physiol.* **150**, 211-216. doi:10.1016/j.cbpa.2008.04.594

Powell, F. L. and Gray, A. T. (1989). Ventilation-perfusion relationships in alligators. *Respir Physiol.* **78**, 83-94. doi:10.1016/0034-5687(89)90144-8

Saltin, B. and Calbet, J. A. L. (2006). Point: in health and in a normoxic environment, $V_{O_2,\mathrm{max}}$ is limited primarilyvby cardiac output and locomotor muscle blood flow. *J. Appl. Physiol.* **100**, 744-748. doi:10.1152/japplphysiol.01395.2005

Schaeffer, P. J., Nichols, S. D. and Lindstedt, S. L. (2007). Chronic electrical stimulation drives mitochondrial biogenesis in skeletal muscle of a lizard, *Varanus exanthematicus*. *J. Exp. Biol.* **210**, 3356-3360. doi:10.1242/jeb.007088

Scheid, P. and Piiper, J. (1980). Blood/gas equilibrium of carbon dioxide in lungs. A critical review. *Respir. Physiol.* **39**, 1-31. doi:10.1016/0034-5687(80)90011-0

Szucsik, A. M., Rourke, B. C., Bennett, A. F. and Hicks, J. W. (2004). The effects of aerobic exercise training on skeletal muscle plasticity in the savannah monitor lizard. *FASEB J.* **18A**, 237.

Taylor, C. R. and Weibel, E. R. (1981). Design of the mammalian respiratory system. I. Problem and strategy. *Respir. Physiol.* **44**, 1-10. doi:10.1016/0034-5687(81)90073-6

Thompson, G. G. and Cowan, E. (1997). Do training and captivity affect maximal metabolic rate of *Varanus gouldii* (Squamata: Varanidae)? *Amphib-Reptilia* **18**, 112-116. doi:10.1163/156853897X00350

Wagner, P. D. (2006). Counterpoint: in health and in a normoxic environment, $V_{O_2,\mathrm{max}}$ is not limited primarily by cardiac output and locomotor muscle blood flow. *J. Appl. Physiol.* **100**, 744-748. doi:10.1152/japplphysiol.01395a.2005

Wang, T. and Hicks, J. W. (2002). An integrative model to predict maximum O_2 uptake in animals with central vascular shunts. *Zoology* **105**, 45-53. doi:10.1078/0944-2006-00043

Wang, T. and Hicks, J. W. (2004). Why savannah monitor lizards hyperventilate during activity: a comparison of model predictions and experimental data. *Respir. Physiol. Neurobiol.* **144**, 251-261. doi:10.1016/j.resp.2004.06.022

Wang, T., Smits, A. W. and Burggren, W. W. (1998). Pulmonary function in reptiles. In *Biology of Reptilia: Morphology G: Visceral Organs*, vol. 19 (ed. C. Gans and A. S. Gaunt), pp. 297-374. SSAR Press.

Wang, T., Joyce, W. and Hicks, J. W. (2019). Similitude in the cardiorespiratory responses to exercise across vertebrates. *Curr. Opin. Physiol.* **10**, 137-145. doi:10.1016/j.cophys.2019.05.007