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SHORT COMMUNICATION

Does the left aorta provide proton-rich blood to the gut when crocodilians digest a meal?

Justin L. Conner[1], Janna L. Crossley[1], Ruth Elsey[2], Derek Nelson[1], Tobias Wang[3] and Dane A. Crossley[1,*]

ABSTRACT

Reptiles have the capacity to differentially perfuse the systemic and pulmonary vascular circuits via autonomic regulation of the heart and the vascular trees. While this aptitude is widely recognized, the role of 'shunting' as a homeostatic mechanism to match convective transport with tissue demand remains unknown. In crocodilians, it has been hypothesized that a pulmonary vascular bypass of systemic venous blood – a right-to-left (R–L) shunt – serves to deliver CO_2-rich blood with protons needed for gastric acid secretion during digestion. This hypothesis is partially based on the unique crocodilian vascular anatomy where a left aorta (LAo) arises from the right ventricle, and appears to preferentially supply the gastrointestinal system, whereas the right aorta emerges from the left ventricle. Recent theoretical considerations imply that a R–L shunt would have minuscule effects on P_{CO_2}, but direct measurements of blood gases in both the right and left aortae or both the right and left atria in fed animals have not been conducted. For this reason, we measured blood parameters including P_{O_2}, P_{CO_2}, pH_e and [HCO_3^-] in the right and left aortae and atria following ingestion of a gavage-fed standardized meal (5% body mass). Blood samples were taken at 3, 6, 12, 24, 36 and 48 h into the digestive period to encompass the period of maximal gastric acid secretion. At no point did P_{CO_2} or pH differ between the left and right aortae, whereas P_{O_2} was significantly lower in the left aorta at several time points during digestion. Our findings do not support the hypothesis that a R–L shunt serves to deliver CO_2 for the gastrointestinal system after feeding in crocodilians.

KEY WORDS: Reptile, Alligator, Digestion, Cardiovascular, Cardiac shunt, Acid–base balance, Blood gases

INTRODUCTION

Crocodilians have a unique cardiovascular design where the left aortic arch (LAo) emerges from the right ventricle and provides an avenue for oxygen-poor and CO_2-rich venous blood to bypass the lungs and re-enter the systemic circulation (White, 1956; Jones, 1996; Axelsson and Franklin, 2011). Under conditions of low systemic arterial blood pressures or decreased pulmonary artery vessel conductance, the LAo contains shunted oxygen-poor blood from the right ventricle, but the LAo also receives arterial O_2-rich blood from the right aorta through a direct aperture in the common wall separating the two aortae (the foramen of Panizza) immediately outside the ventricle in some undisturbed crocodilians (Sabatier, 1883; White, 1956; Axelsson and Franklin, 2001, 2011). The right-to-left (R–L) shunt occurs when pressure in the right ventricle exceeds the systemic arteries and its magnitude is under exquisite regulation of the autonomic nervous system (Webb, 1979; Jones and Shelton, 1993; Franklin and Axelsson, 2000; Axelsson and Franklin, 2001).

The left and the right aortae of crocodilians merge distal to the heart through an anastomosis, and the left aorta continues as the coeliac artery (Reese, 1915; Farmer, 2011). It appears therefore that stomach, pancreas, liver and proximal small intestine are supplied with CO_2-rich and oxygen-poor blood, whilst the right aorta supplies the mesenteric, urogenital and posterior somatic tissue (Reese, 1915; Farmer, 2011). The peculiar vascular anatomy of crocodilians has spurred the suggestion that shunting of CO_2-rich blood into the LAo is beneficial for gastric acid secretion, where the protons secreted by the parietal cells stem from hydration of CO_2 by carbonic anhydrase (Jones and Shelton, 1993; Farmer et al., 2008; Gardner et al., 2011; Farmer, 2011). Consistent with this proposal, chronic ligation of the LAo prolonged digestion of bone meals and appeared to be associated with decreased gastric acid secretion in alligators (Farmer et al., 2008). Furthermore, blood flows in the LAo and the celiac artery increase during digestion in crocodilians (Axelsson et al., 1991; Farmer et al., 2008; Findsen et al., 2018), but it remains unknown whether the blood conveyed by the LAo is indeed derived from the right ventricle during digestion. Further ligation of the LAo has no impact on overall growth rates of American alligators (Eme et al., 2010). The fundamental hypothesis of the R–L shunt serving to deliver CO_2-rich blood to digestive organs was recently questioned on theoretical grounds. Based on mathematical modeling, Malte et al. (2017) argued that the R–L shunt would be rather ineffective at elevating arterial partial pressure of CO_2 (P_{CO_2}), and that an increased R–L shunt would compromise oxygen delivery during digestion. Instead, it was reasoned that the hypoventilation, which characterizes the postprandial state (Wang et al., 2001), would be a much more effective mechanism to elevate arterial P_{CO_2} with minimal effect on arterial oxygen levels (Malte et al., 2017).

To resolve this controversy, we aimed to determine blood gas composition in the LAo and RAo during fasting and digestion in alligators with the specific goal of investigating whether P_{CO_2} of the LAo is elevated during the postprandial period. In a separate group of animals, we performed similar measurements in both atria to address the influence of digestion on venous blood gases.

MATERIALS AND METHODS

Experimental animals

American alligators (*Alligator mississippiensis* Daudin 1802) were hatched from eggs that had been collected from the

[1]Department of Biological Sciences, University of North Texas, Denton, TX 76203, USA. [2]Department of Wildlife and Fisheries, Grand Chenier, LA 70603, USA. [3]Zoophysiology, Department of Bioscience, Aarhus University, 8000 Aarhus C, Denmark.

*Author for correspondence (dane.crossley@unt.edu)

D.A.C., 0000-0001-9683-7013

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Rockefeller Wildlife Refuge in Grand Chenier, LA, USA in the summer of 2014 and transported to the University of North Texas (Denton, TX, USA). The eggs were incubated at 30°C in 3.5% CO_2 under conditions previously described as part of a larger embryonic study (Eme and Crossley, 2015). Upon hatching, alligators were maintained at 24°C in tanks (50–500 l) with access to dry land and fresh water. Animals were fed Crocodilian Diet (Mazuri, PMI Nutrition International, Brentwood, MO, USA) *ad libitum* four times per week, and kept on a 12 h light:12 h dark cycle (light: 08:00–20:00 h). The animals used in this study were 2.5 to 3 years old. All procedures and studies were approved by UNT IACUC protocol #17-001.

Surgical procedures

Two studies were performed on two separate groups of alligators that had been fasted for 18–20 days. To induce anesthesia, a plastic container containing isoflurane-saturated cotton gauze (Isoflo, Abbott Laboratories, North Chicago, IL, USA) was placed over the head. The animal was then placed supine and intubated with soft Tygon tubing for mechanical ventilation (7–10 breaths min^{-1} and a tidal volume of 25 ml kg^{-1}) with 1% isoflurane in room air using a Harvard Apparatus 665 ventilator (Harvard Apparatus, Holliston, MA; FluTec vaporizer, FluTec, Ohmeda, OH, USA). Reflexes were monitored regularly to ensure a surgical plane of anesthesia.

After cleaning the ventral surface, lidocaine (4.5 mg kg^{-1} Lidoject, Henry Schein, Dublin, OH, USA) 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 partially split at the midline with a scalpel blade (size 22), to access the pericardium and the major arteries.

In seven individuals, the left and right aortae were isolated by blunt dissection. Having passed silk suture (4-0) under each vessel, flows were briefly interrupted, so a PE 10 catheter (Polyethylene tubing, Braintree Scientific, Braintree, MA, USA) with a heat-flared tip could be inserted non-occlusively through a small incision. The catheters were secured to the vessel wall with 6-0 silk using a purse stitch. Each PE 10 catheter was 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) and a CA bonding accelerant (Zip Kicker, Pacer Technology, Ontario, CA, USA).

In another seven individuals, both atria were exposed through 0.5–1 cm incisions in the pericardium and retracted. A small hole (<0.5 cm) was cut to allow insertion of one PE 50 catheter with bubbles at the tip into each atrium. Atrial tissue was fastened around the catheter with two 4-0 sutures and the pericardium was closed with 5-0 silk. For both procedures, the catheters were tunneled under the skin and externalized through a dorsal perforation. The sternum and overlying skin were closed by sutures and catheters were secured to the back of the animal.

Feeding protocol and post-feeding blood parameter measurements

Both groups of animals recovered in plastic containers (50×40×40 cm) for 24 to 36 h in an environmental room at 30°C and were treated with 1.1 mg kg^{-1} analgesic (FluMeglumine, Flunixin Meglumine, Clipper Distributing Company, St Joseph, MO). During this time, animals were exposed to their normal 12 h light:12 h dark cycle. Following recovery, control blood samples (designated as time 0) were drawn from fasting animals, and the alligators were now gavage fed ∼5% of their body mass of a hydrated dry-pellet paste (40% Mazuri Crocodilian Diet, 60% water), and animals were visually checked every hour post-feeding prior to blood sampling to ensure that food was not regurgitated. The animals were then returned to their plastic containers, the catheters suspended by helium balloons, such that blood samples could be drawn at 3, 6, 12, 24, 36, 48 h post-feeding without disturbing the alligators. All animals were euthanized by a vascular injection of sodium pentobarbital (150 mg kg^{-1}) upon completion of measurements.

Measurement of blood gases

Blood samples (500 µl) were drawn simultaneously from each of the two cannulae to determine 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. Hematocrit was measured upon centrifugation (20,854 ***g*** for 5 min at Micro-Hematocrit Damon/IEC Division), and blood glucose and lactate concentrations were determined (2300 STAT Plus, YSI, Yellow Springs, OH, USA). Plasma osmolarity was measured with a Wescor osmometer (Vapro Model 5600, Logan, UT, USA).

Statistical analysis

The influence of digestion on blood gases was analyzed with an ANOVA for repeated measures (Statistica v13; StatSoft, Tulsa, OK, USA). Fisher's LSD *post hoc* tests were used for pairwise comparisons between time points post-feeding. An unpaired Student's *t*-test was used to determine differences in the blood parameters between the right and left aortae. The alpha level for these *t*-tests was Bonferroni corrected by dividing by two, accounting for data use to test multiple differences. This method was selected to account for periodic missing data in the data set due to sampling errors or equipment failure. These methods were also used to test for differences in blood parameters following feeding and between the atria. Comparisons between values in the aortae and the atria were not conducted because these studies were conducted on different sets of experimental animals. Data are presented as means±s.e.m., with statistical significance being designated when $P<0.05$.

RESULTS AND DISCUSSION

The blood samples drawn from undisturbed fasting alligators demonstrated a low P_{O_2} in the right atrium compared with the left atrium (43.7±4.0 and 81.3±3.3 mmHg, respectively), whilst P_{O_2} in RAo and LAo was rather similar (80.9±2.9 and 73.7±6.4 mmHg; Fig. 1A,D). Consistent with the much higher solubility of CO_2 compared with oxygen and the fact that most of the CO_2 is transported as HCO_3^-, primarily in plasma or bound to the unique crocodilian hemoglobin (Bauer and Jelkmann, 1977; Grigg and Cairncross, 1980; Weber and White, 1986; Jensen et al., 1998), there were much smaller differences between the P_{CO_2} in the atria and aortae. Thus, P_{CO_2} of fasting alligators was 31.9±2.1 and 31.1±2.1 mmHg in the right and left atria, respectively, and 33.7±1.7 and 36.0±1.5 mmHg in the RAo and LAo, respectively (Fig. 1B,E). pH was around 7.40–7.45 and similar at all sampling sites (Fig. 1C,F). All blood gases of the fasting alligators were in accordance with earlier measurements of arterial blood from indwelling catheters in the femoral or carotid arteries, i.e. blood derived from the RAo (Seymour et al.,

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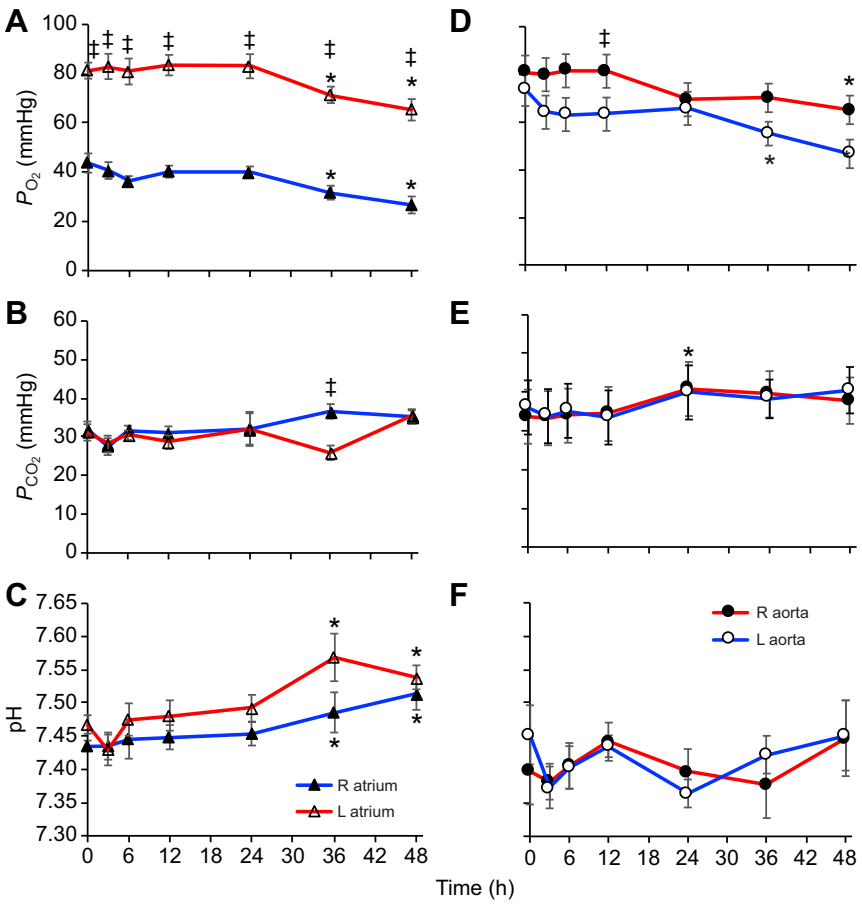

Fig. 1. Partial pressure of gases and pH of blood sampled during digestion in American alligators. Partial pressure of oxygen in blood (P_{O_2}), partial pressure of CO_2 (P_{CO_2}) and blood pH in seven alligators with indwelling catheters in left and right atria (A–C) and another group of seven individuals with indwelling catheters in the left and right aortae (LAo and RAo, respectively) (D–F). Samples were drawn from fasting animals (time 0) and at 3, 6 12, 24, 36 and 48 h into the postprandial period after gavage feeding with a meal of 5% of body mass. Asterisks indicate a difference from fasting values based on a repeated-measures ANOVA; ‡ indicates a difference between the two sampling sites based on the unpaired *t*-test ($P<0.05$). All data are presented as means±s.e.m. with a sample size of 7 in each group.

1985; Busk et al., 2000; Hartzler et al., 2006), as well as samples drawn from chronic catheters in the atria of alligators (Hicks and White, 1992). The placement of the catheters for blood sampling from the central circulation required extensive surgery, and the opening of the sternum is likely to have imposed discomfort on the animals, but given that all blood gas results in fasting and digesting animals are similar to published data, we believe that the animals exhibited normal physiological responses to digestion.

Digestion was associated with a small rise in pH at 36 and 48 h into the postprandial period, and this ‘alkaline tide’ was attended by a rise in arterial P_{CO_2} (statistically significant in only the RAo at 24 h; Fig. 1E). The respiratory compensation and the magnitude of the metabolic alkalosis was similar, but less pronounced than that described by Busk et al. (2000) for the same species, but clearly shows that gastric acid secretion did elicit a typical ‘alkaline tide’ (Wang et al., 2001). Feeding caused significant reductions in P_{O_2} of the aortae and the atria and P_{O_2} in the RAo tended to be higher than the LAo throughout digestion (significant only at 12 h; Fig. 1D). The elevated P_{O_2} in the RAo, while right atrium P_{O_2} deceased after feeding, suggests an increase in atrioventricular difference. There were no changes in plasma osmolarity (Table 1), and the initial small rise in blood lactate probably stems from handling during gavage feeding (Table 2; Seymour et al., 1985). Plasma blood glucose was also elevated in the atria during digestion (Table 2).

The similarity of oxygen levels in the two aortae agrees with previous studies on chronically cannulated crocodilians (White, 1956; Greenfield and Morrow, 1961; Khalil and Zaki, 1964; Grigg and Johansen, 1987). However, there was a difference at 48 h, which demonstrates that the blood conveyed by the LAo primarily

Table 1. Hematocrit and plasma osmolarity in blood from aortae and atria of fasted and fed alligators

	Hct (%)				Osm (mmol kg^{-1})			
	Right aorta	Left aorta	Right atrium	Left atrium	Right aorta	Left aorta	Right atrium	Left atrium
Fasting	20.9±0.7 (7)	21.8±1.3 (7)	24.6±0.7 (7)	24.5±0.5 (7)	275±7 (7)	278±4 (7)	286±5 (6)	279±6 (6)
3 h	19.4±1.1 (7)	19.4±1.0 (7)	24.7±1.4 (7)	24.0±0.9 (7)	274±8 (7)	274±5 (7)	279±7 (6)	291±8 (6)
6 h	18.3±1.2 (7)	19.4±0.8 (7)	23.5±1.3 (7)	22.5±1.4 (7)	274±4 (7)	278±4 (7)	279±7 (6)	274±10 (6)
12 h	18.7±1.3 (7)	18.2±1.4 (7)*	24.9±0.9 (6)	24.5±1.4 (6)	280±6 (7)	279±5 (7)	272±16 (5)	277±5 (6)
24 h	18.0±1.6 (7)	18.2±1.9 (7)*	23.3±2.4 (6)	23.2±2.4 (6)	274±4 (7)	271±5 (7)	284±5 (6)	283±6 (6)
36 h	17.2±2.2 (6)	16.7±2.4 (5)*	21.5±2.6 (6)	21.6±3.3 (6)	274±7 (6)	271±8 (5)	272±6 (6)	278±6 (6)
48 h	14.3±2.8 (6)	13.97±2.4 (6)*	20.6±3.3 (6)	20.1±2.8 (6)	263±7 (6)	261±8 (6)	284±6 (6)	280±7 (6)

Blood samples were taken at indicated times after feeding to measure hematocrit (Hct) and plasma osmolarity (Osm). Data are presented as means±s.e.m. In all cases, sample size is indicated in parentheses. *$P<0.05$, compared with fasting level.

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Table 2. Lactate and glucose levels measured in blood taken from the aortae and atria of fasted and fed alligators

	Lactate (mmol l^{-1})				Glucose (mmol l^{-1})			
	Right aorta	Left aorta	Right atrium	Left atrium	Right aorta	Left aorta	Right atrium	Left atrium
Fasting	0.37±0.06 (7)	0.45±0.10 (7)	1.00±0.32 (7)	0.61±0.12 (7)	5.17±0.75 (7)	5.96±1.03 (7)	3.25±0.17 (7)	3.03±0.30 (7)
3 h	2.00±0.67 (7)*	1.84±0.64 (7)	2.36±0.68 (7)	2.52±0.71 (7)*	6.18±0.56 (7)	5.87±0.77 (7)	4.49±0.59 (7)*	4.88±0.43 (7)*
6 h	1.45±0.58 (7)*	1.44±0.63 (7)	1.52±0.41 (7)	1.52±0.46 (7)	6.62±0.74 (7)	5.99±0.60 (7)	5.06±0.52 (7)*	4.95±0.48 (7)*
12 h	1.10±0.52 (7)	1.20±0.52 (7)	1.25±0.35 (6)	1.32±0.35 (6)	5.94±0.58 (7)	6.36±0.41 (7)	5.40±0.65 (6)*	5.03±0.39 (6)*
24 h	1.24±0.49 (7)	1.43±0.47 (7)	1.91±0.90 (6)	1.90±0.78 (6)	6.81±0.91 (7)	6.73±0.87 (7)	5.30±0.81 (6)*	5.51±0.70 (6)*
36 h	1.61±0.64 (5)*	1.51±066 (5)	1.15±0.11 (6)	1.04±0.15 (6)	6.21±0.96 (6)	6.41±1.26 (5)	5.33±0.73 (6)*	5.15±0.75 (6)*
48 h	0.80±0.20 (6)	0.84±0.17 (6)	1.42±0.35 (6)	1.26±0.28 (6)	5.61±1.18 (6)	6.12±0.71 (6)	5.28±0.69 (6)*	5.00±0.62 (6)*

Blood samples were taken at indicated times after feeding to measure lactate and glucose levels. Data are presented as means±s.e.m. In all cases, sample size is indicated in parentheses. *P<0.05, compared with fasting level.

stems from the left ventricle, suggesting that there is a R–L shunt during digestion. Shunt flows in fully recovered crocodilians, at ∼10–25% of cardiac output, have also been measured or inferred from pressure and flow measurements (e.g. Axelsson et al., 1989; Jones and Shelton, 1993; Findsen et al., 2018). The P_{O_2} difference between the RAo and LAo widened at most time points into the postprandial period, and may indicate a modest rise in the amount of blood shunted from the right ventricle to the LAo. This is consistent with recent blood flow measurements in digesting alligators (Findsen et al., 2018).

The aim of this study was to investigate whether the LAo preferentially provides for CO_2-rich blood to the gastrointestinal tract during digestion. Based on the dependence of gastric acid secretion by the parietal cells on protons formed from hydration of CO_2, it has been suggested that the shunting of CO_2-rich blood into the LAo incites digestion, and it does appear that gastric degradation of a bone meal is prolonged in alligators with chronic ligation of the LAo (Farmer et al., 2008). However, based on first principles, we recently developed a model to predict the influence of a R–L shunt, ventilation and metabolism on blood gases, and demonstrated that rather large R–L shunts would be required to markedly increase arterial P_{CO_2} (Malte et al., 2017). Importantly, this model predicts that the required R–L shunt flows needed to elevate P_{CO_2} would markedly reduce arterial P_{O_2} and O_2 concentration, whereas a similar rise in P_{CO_2} could be attained by means of proportional hypoventilation (i.e. a decrease in ventilation relative to metabolic CO_2 production) with minimal effects on arterial oxygen levels (Malte et al., 2017). As predicted by such a model, and as a simple consequence of the much higher solubility of CO_2 compared with oxygen in blood, our measurements here of arterial blood gases clearly demonstrate differences in P_{CO_2} in the RAo between fasting and digesting alligators 24 h after feeding. If the blood gas data were used to calculate the differences (between RAo and LAo) in blood gases and then plotted as ΔP_{CO_2} versus ΔP_{O_2} (Fig. 2), in some measurements there was up to a 15 torr increase in LAo P_{CO_2} compared with the RAo; however, this value was associated with a 35 torr reduction in P_{O_2} in blood that supplies the gut (Fig. 2). In this analysis, we have also included similar measurements by Grigg and Johansen (1987) on fasting *Crocodylus porosus*, where blood gas data and pressure recording allowed for predictions of whether the crocodiles were shunting during blood sampling. As represented in Fig. 2, increases in P_{CO_2} in the LAo, by means of a R–L shunt, are accompanied by a larger reduction in P_{O_2} in the LAo. Given the known increase in aerobic metabolism associated with digestion in crocodilians, it seems that increasing LAo P_{CO_2} at the cost of P_{O_2} would constrain the digestive process. Thus, if gastric acid secretion, pancreatic base secretion and intestinal base secretion are indeed influenced by physiologically relevant changes in P_{CO_2} of the arterial supply, we surmise that the hypoventilation that occurs in all digesting air-breathing vertebrates, fully suffices in elevating P_{CO_2} in a manner that also provides pH homeostasis for all organs and tissues. Our findings failed to support the hypothesis proposing that LAo blood contains elevated P_{CO_2} during digestion.

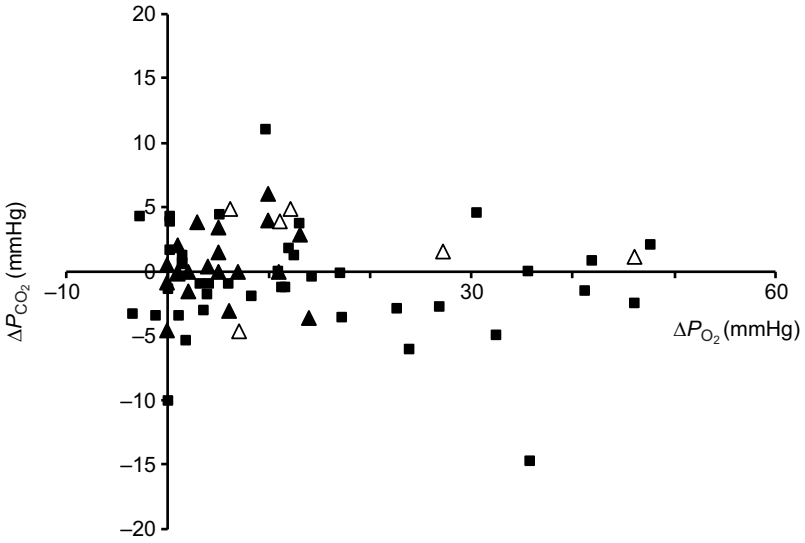

Fig. 2. Differences in P_{CO_2} and P_{O_2} between blood drawn simultaneously from RAo and LAo in American alligators. All individual samples from the present study are shown as black squares. The figure also includes similar data reported by Grigg and Johansen (1987) for *Crocodylus porosus* (black triangles); open triangles indicate samples where Grigg and Johansen (1987) concluded that the crocodiles exhibited R–L shunt based on pressure and blood gas measurements.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: J.L. Crossley, T.W., D.A.C.; Methodology: J.L. Conner, J.L. Crossley, D.N., T.W., D.A.C.; Formal analysis: J.L. Conner, D.N., T.W., D.A.C.; Investigation: D.A.C.; Resources: J.L. Conner, R.E., D.A.C.; Data curation: D.A.C.; Writing - original draft: J.L. Conner, R.E., D.N., T.W., D.A.C.; Writing - review & editing: J.L. Conner, R.E., D.N., T.W., D.A.C.; Supervision: D.A.C.

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