

Terrestrial locomotion does not constrain venous return in the American alligator, *Alligator mississippiensis*

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Summary

The effects of treadmill exercise on components of the cardiovascular (heart rate, mean arterial blood pressure, central venous pressure, venous return) and respiratory (minute ventilation, tidal volume, breathing frequency, rate of oxygen consumption, rate of carbon dioxide production) systems and on intra-abdominal pressure were measured in the American alligator, *Alligator mississippiensis*, at 30°C. Alligators show speed-dependent increases in tidal volume and minute ventilation, demonstrating that the inhibition of ventilation during locomotion that is present in some varanid and iguanid lizards was not present in alligators. Exercise significantly increases intra-abdominal pressure; however, concomitant elevations in central venous pressure acted to increase the transmural pressure of the post caval vein and thus increased venous return. Therefore, despite elevated intra-

abdominal pressure, venous return was not limited during exercise in alligators, as was the case in *Varanus exanthematicus* and *Iguana iguana*. Respiratory cycle variations in intra-abdominal pressure, central venous pressure and venous return indicate that, at high tidal volumes, inspiration causes a net reduction in venous return during active ventilation and thus may act to limit venous return during exercise. These results suggest that, while tonically elevated intra-abdominal pressure induced by exercise does not inhibit venous return, phasic fluctuations during each breath cycle may contribute to venous flow limitation during exercise.

Key words: exercise, hemodynamics, intra-abdominal pressure, locomotion, oxygen consumption, reptile, venous return, ventilation, central venous pressure, heart rate.

Introduction

Recent studies have demonstrated that limitations to oxygen transport in lizards occur within both the respiratory and cardiovascular systems. The lateral flexions of the trunk that occur during locomotion in lizards result in a speed-dependent constraint on lung ventilation (Carrier, 1987; Owerkowicz et al., 1999; Wang et al., 1997). This constraint (termed axial constraint) results from the conflict between lateral undulation, requiring unilateral recruitment of the trunk muscles, and costal ventilation, requiring bilateral recruitment of the same trunk muscles. Some species of varanid lizards overcome this constraint by employing an accessory ventilatory mechanism called the gular pump, thus maintaining oxygen consumption despite decreasing costal ventilation (Munns et al., 2004; Owerkowicz et al., 1999).

Locomotion increases intra-abdominal pressure (IAP) in *Varanus exanthematicus* (Munns et al., 2004). High IAP (~20 mmHg; 1 mmHg=133.3 Pa), both at rest and during exercise, was associated with decrements in post caval blood flow (\dot{Q}_{PC}) via decreases in transmural pressure. Elevated IAP associated with decrements in \dot{Q}_{PC} have also been measured during exercise in *Iguana iguana* (Farmer and Hicks, 2000).

Elevated IAP can act to limit venous return when IAP exceeds central venous pressure, causing partial or complete collapse of the main veins in the abdominal compartment. Systemic venous return and ventricular preload are major determinants of cardiac output, and thus reductions in venous return have important impacts on oxygen delivery during exercise.

In contrast to the sprawling gait used by lizards (Farley and Ko, 1997), crocodylians use a semi-erect posture during locomotion ('high walk') in which the body is held in an intermediate position between a sprawling and an erect gait (Reilly and Blob, 2003; Reilly and Elias, 1998). Despite the differences in gait between lizards and crocodylians, both groups experience a substantial degree of lateral trunk bending during locomotion (Farley and Ko, 1997; Reilly and Elias, 1998). In addition, crocodylians possess significantly different ventilatory mechanics compared with lizards. In crocodylians, the lateral and ventral portions of the liver are attached to the pelvis by a diaphragmaticus muscle (Gans and Clark, 1976b; Naifeh et al., 1970a, 1971). Contraction of the diaphragmaticus muscle retracts the liver caudally, increasing

pleural cavity volume and thus effecting lung inflation (Farmer and Carrier, 2000a; Gans and Clark, 1976; Grigg and Gans, 1993a). The intercostal musculature is also active during inspiration (Gans and Clark, 1976), as are the ischiopubis and ischiotruncus muscles, which act to expand the abdomen by expansion of the ribs and ventral rotation of the pubic bones, respectively (Farmer and Carrier, 2000a). By contrast, expiration is caused by contractions of the superficial intercostal (Gans and Clark, 1976), the transverse abdominal (Farmer and Carrier, 2000a; Gans and Clark, 1976) and the rectus abdominus muscles (Farmer and Carrier, 2000a), which move the liver anteriorly and reduce the volume of the abdominal cavity (Farmer and Carrier, 2000a; Grigg and Gans, 1993a). Thus, both inspiration and expiration are active in crocodiles (Farmer and Carrier, 2000a; Naifeh et al., 1971), and lung ventilation can be effected solely by use of the hepatic piston pump (Gans and Clark, 1976) or by use of costal ventilation (Hartzler et al., 2004) or by a combination of both (Farmer and Carrier, 2000a).

An important consequence of the ventilatory mechanics in alligators is the separation of the muscles used for ventilation and locomotion. Activity of the diaphragmaticus, transversus abdominus, rectus abdominus and ischiopubis muscles is tightly correlated with the respiratory cycle in exercising alligators, but these muscles are only intermittently active or are inactive during locomotion (Farmer and Carrier, 2000a). Thus, despite alligators engaging in lateral bending of the trunk during locomotion and having a similar posture to that of lizards (factors predisposing them to ventilatory limitations during exercise), alligators retain the ability to run and breathe at the same time (Farmer and Carrier, 2000a). The lack of axial constraint in alligators may result in the generation of lower abdominal pressures and a reduced venous flow limitation during exercise.

The aim of this study was to determine the effects of treadmill exercise on aspects of the cardiovascular and respiratory systems in the American alligator, *Alligator mississippiensis*. We hypothesized that, due to the absence of axial constraint in crocodylians, any increase in IAP will be exceeded by elevations in central venous pressure, resulting in no suppression of venous return during exercise.

Materials and methods

Animals

Alligators [*Alligator mississippiensis* (Daudin 1802)] were obtained from the Rockefeller Wildlife Center, Louisiana, and kept in aquaria with a thermal gradient (27–33°C), full spectrum lighting (14 h:10 h L:D; Zoo Med, San Luis Obispo, CA, USA) and free access to water and were fed a diet of chicken pieces, whole rodents and fish. The body masses of the five alligators (three females and two males) used increased during their captivity (from 2.26±0.28 kg to 2.84±0.43 kg; means ± S.E.M.) and ranged from 1.67 to 3.60 kg at the time of experimentation.

Surgical procedure, blood flow, intra-abdominal pressure and blood pressure

The surgical procedures used in this study are presented in detail for Savannah monitor lizards (*Varanus exanthematicus*) in Munns et al. (2004) and are briefly described below. Alligators were lightly anesthetized by placing them in a sealed container with gauze dampened with isoflurane (Isoflo; Abbott Laboratories, North Chicago, IL, USA). Alligators were then intubated and artificially ventilated (SAR-830; CWE Inc., Ardmore, PA, USA) with room air that had been passed through a vaporizer (Dräger, Lubeck, Germany). The vaporizer was initially set at 3–4% and was then reduced to 1–2% for the majority of the surgery. A 3–5 cm ventral incision was made in the abdomen, and two loose-fitting ultrasonic blood flow probes (2R; Transonic System Inc., Ithaca, NY, USA) were placed around each post caval vein (homologous to the mammalian inferior vena cava). In *Alligator mississippiensis*, the two post caval veins carrying blood from the hindlimbs, tail and pubic region do not fuse prior to entry into the liver; instead, each post caval vein enters ipsilateral lobes of the liver directly. Each ultrasonic blood flow probe was placed around one post caval vein anterior to the kidney but posterior to its entry into the liver. The intestinal, lienogastric, gastric and abdominal veins fuse to form the hepatic portal vein, which enters the left lobe of the liver (Schaffner, 1998). Thus, the venous return measured in this study comprises venous drainage from the hindlimbs and tail but excludes drainage from the gastrointestinal tract, which enters the liver anterior to the location of the blood flow probe. IAP was measured using a pressure transducer (Millar Mikrotip; Millar Instruments, Inc., Houston, TX, USA) sutured to connective tissue adjacent to the flow probes. The probes were exteriorized through the lateral body wall approximately 5 cm anterior to the pelvis and secured using 3-0 silk sutures on the dorsal surface of the tail.

The femoral artery was cannulated in order to measure arterial blood pressure. A 1–2 cm incision was made in the ventral surface of the hindlimb. The femoral artery was exposed using blunt dissection techniques, and the artery cannulated with polyethylene tubing (I.D. 0.023 cm, O.D. 0.038 cm; Harvard Apparatus, Inc., Holliston, MA, USA) and secured using 3-0 sutures. Blood pressure was measured using disposable pressure transducers (model MLT0670; ADInstruments, Colorado Springs, CO, USA). The contralateral femoral vein was also cannulated to facilitate measurement of central venous pressure. A 3.5F pressure transducer (Millar Mikrotip; Millar Instruments, Inc.) was introduced into the femoral vein and advanced 8–10 cm into the central venous circuit.

All incisions were closed with interrupted sutures and treated with cyanoacrylate tissue adhesive (Vetbond; 3M, St Paul, MN, USA). Artificial ventilation with room air was continued until the alligator regained consciousness and reinitiated spontaneous breathing. Intramuscular injections of the antibiotic enrofloxacin (Baytril; Bayer Corporation, Shawnee Mission, KS, USA) and the analgesic flunixin

meglumine (Flunixinamine; Fort Dodge, Madison, NJ, USA) were given at the conclusion of surgery. Enrofloxacin injections were repeated every second day after surgery. A minimum recovery period of two days was given before commencement of experimentation.

Lung ventilation and gas exchange

Ventilation was measured using a mask constructed from the base of a 50 ml polypropylene centrifuge tube (Corning Inc. Life Sciences, Acton, MA, USA). Flexible tubing was attached *via* two ports drilled into the mask. The mask was attached over the alligator's nostrils, and the mouth sealed closed with a dental polyether impression material (Impregum F; 3M). Fresh room air was drawn through the mask using a sealed aquarium air pump at a constant flow rate of 1.2–1.7 l min⁻¹ (depending on the size of the alligator). Care was taken to ensure that the flow rate through the mask exceeded the rate of inspiration, thus minimizing the possibility of rebreathing. Air flow through the mask was controlled with rotameters (Brooks Instruments, Hatfield, PA, USA). Alterations in airflow due to ventilation were measured using a pneumotachograph (8311; Hans Rudolph, Inc., Kansas City, MO, USA) placed upstream of the mask, such that expirations caused an increase in airflow and inspiration caused a decrease in airflow. Pressure gradients induced by alterations in airflow across the pneumotachograph were monitored using a differential pressure transducer (MP-45-1-871; Validyne, Northridge, CA, USA). The signal from the differential pressure transducer was calibrated by injecting and withdrawing known volumes of gas from the sealed mask and was integrated to obtain tidal volumes. Gas from the mask was sub-sampled and passed through Drierite[®] (anhydrous calcium sulfate) before being passed through CO₂ (CD-3A; Applied Electrochemistry, Inc., Sunnyvale, CA, USA) and O₂ (S-3A; Applied Electrochemistry, Inc.) analyzers. The rates of oxygen consumption (\dot{V}_{O_2}) and carbon dioxide production (\dot{V}_{CO_2}) were determined using a technique previously described (Bennett and Hicks, 2001; Farmer and Hicks, 2000; Wang et al., 1997). Briefly, \dot{V}_{O_2} and \dot{V}_{CO_2} of single breaths were determined as the area below (\dot{V}_{O_2}) or above (\dot{V}_{CO_2}) the baseline signal for room air. Exhalations were simulated by injecting known volumes of known gas mixtures (21% O₂, 79% N₂; 100% N₂; 15% O₂, 5% CO₂, 80% N₂) into the mask to establish the relationship between this area and gas exchange. Minute ventilation and tidal volume are reported at BTPS (body temperature and pressure, saturated) and metabolic gas values at STPD (standard temperature and pressure, dry).

Experimental protocol

Alligators were accustomed to walking and running on the treadmill in a series of 'training' bouts for a minimum of 3 months prior to experimentation. Training bouts consisted of alligators repeatedly walking and running on the treadmill at each of the experimental speeds (0.75, 1.0 and 1.5 km h⁻¹) until exhaustion. Training bouts occurred three times a week and continued until all alligators were able to maintain 4 min of

consistent locomotion at each treadmill speed (non consecutive). Alligators were fasted for 7 days prior to surgery and were held at the experimental temperature (30°C) for 2–3 days prior to experimentation. A mask was attached over the alligator's nostrils and the alligator was placed on the treadmill belt. The alligator was left on the stationary treadmill belt for at least one hour before the treadmill was started to obtain pre-exercise 'resting' measurements. All alligators rested quietly on the treadmill during the pre-exercise period. The exercise regime consisted of 4 min exercise bouts at each of three treadmill speeds; 0.75, 1.0 and 1.5 km h⁻¹; each 4 min exercise bout was separated by a minimum of 1 h at rest (during which all experimental parameters returned to pre-exercise values). Locomotion was initiated by gently tapping the treadmill belt behind the alligator or by lightly touching the alligator's tail. Ventilatory and cardiovascular parameters reached a steady state by 3 min of treadmill exercise at each speed.

Data collection, analysis and statistics

All signals were collected on a computer at 100 Hz using Acknowledge data acquisition software (Biopac, Goleta, CA, USA). Due to the intermittent and variable nature of reptilian ventilation and the low breathing frequencies employed at rest, ventilatory and cardiovascular parameters and intra-abdominal pressure were calculated from the last 10 min of the pre-exercise period prior to commencement of each exercise bout. Ventilation, \dot{V}_{O_2} , \dot{V}_{CO_2} , IAP, central venous pressure (CVP) and \dot{Q}_{PC} were calculated from the last minute of treadmill exercise at each speed. Mean arterial blood pressure and heart rate for the exercise period were calculated from the first 10 s of the recovery period after each treadmill speed to avoid any interference caused by the alligator's locomotion. All recovery period data were calculated from a 60 s window 2 min after exercise ceased. Total \dot{Q}_{PC} was calculated from the sum of the left and right post caval flows.

The effect of increasing treadmill speed on all parameters was determined using paired Dunnett's tests with the rest period as the control ($P < 0.05$). Comparisons of the recovery period with the maximal treadmill speed were performed using a paired *t*-test ($P < 0.05$). All data presented are means \pm S.E.M.

Results

Post caval blood flow (\dot{Q}_{PC}) and intra-abdominal pressure (IAP) show variations during the breathing cycle (Fig. 1). Ventilation commences with an expiration, during which IAP decreases and \dot{Q}_{PC} increases. Initially, IAP falls during inspiration, followed by an increase above that measured during the non-ventilatory period. Inspiration is associated with a decrease in \dot{Q}_{PC} . Increasing tidal volumes are negatively correlated with a decrease in \dot{Q}_{PC} ($r^2 = -0.71$, $P < 0.0001$) and positively correlated with IAP ($r^2 = 0.72$, $P < 0.0001$; Fig. 2). Expiration is associated with an increase in \dot{Q}_{PC} ; however, this increase is not significantly correlated with increments in tidal volume ($r^2 = -0.15$, $P = 0.33$; Fig. 2A). The net effect of breath

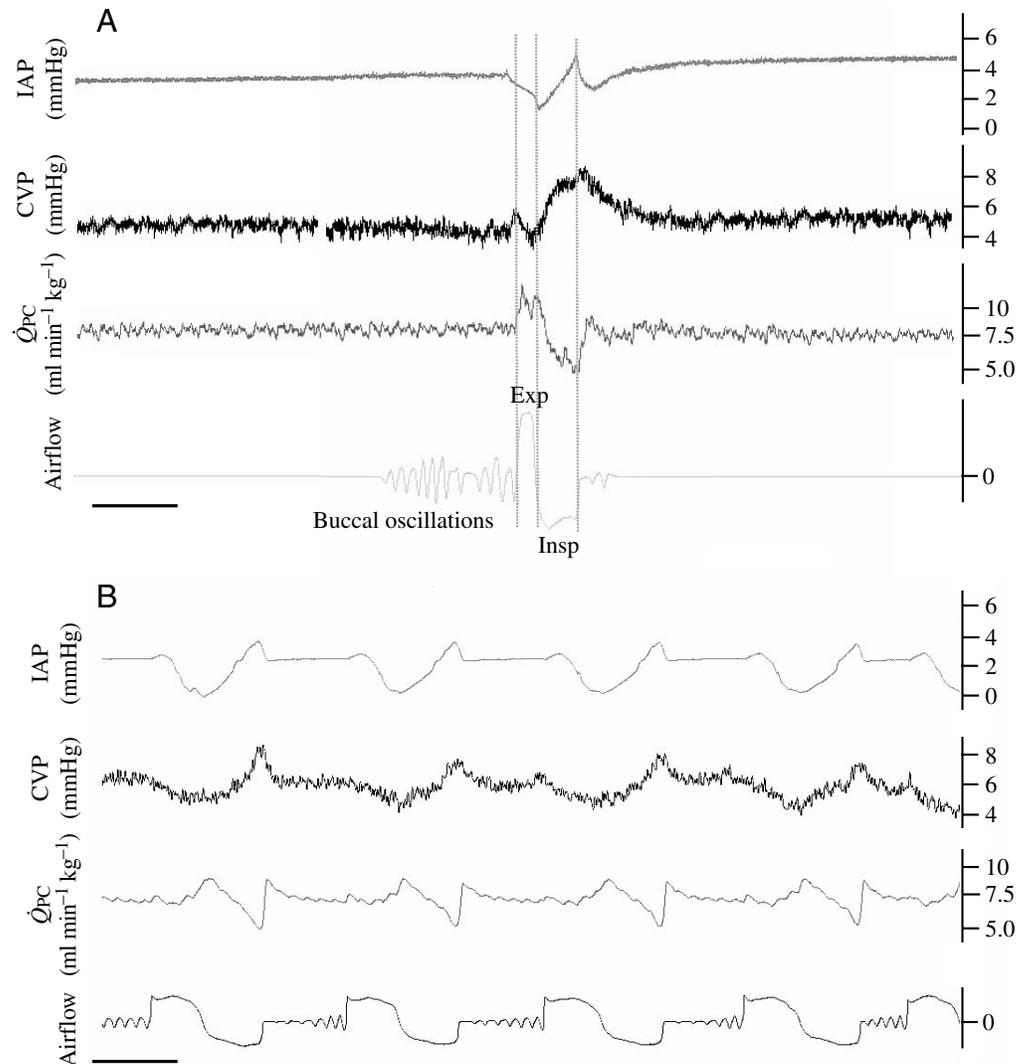


Fig. 1. A sample recording of the variations in intra-abdominal pressure (IAP), central venous pressure (CVP) and total post caval vein blood flow (\dot{Q}_{PC}) during ventilation (A) at rest and (B) running at 1.0 km h⁻¹. During expiration (Exp), IAP falls to near atmospheric pressure, and \dot{Q}_{PC} increases. Peak IAP and CVP and minimum \dot{Q}_{PC} occur at the end of inspiration (Insp). Inspired tidal volume was twofold higher during exercise (46.23 ml kg⁻¹) compared with rest (24.68 ml kg⁻¹). Buccal oscillations (movement of air in and out of the buccal region) occur in alligators but do not contribute to gas exchange. Data are from one alligator (mass 3.6 kg) and are representative of the trend in all alligators tested. Scale bar in A, 15 s; B, 5 s.

cycle variations on \dot{Q}_{PC} is negatively correlated with increments in tidal volume ($r^2 = -0.57$, $P < 0.0001$; Fig. 2B).

Treadmill exercise induced a rapid increase in \dot{Q}_{PC} (Fig. 3). Maximal \dot{Q}_{PC} was achieved within the first 20 s after the onset of exercise at 0.75 km h⁻¹, after which there was no further significant increase in \dot{Q}_{PC} . Maximal \dot{Q}_{PC} was achieved within 40 s after the onset of exercise at both 1.0 and 1.5 km h⁻¹. \dot{Q}_{PC} decreased slowly during the recovery period after exercise and was still significantly elevated compared with resting levels 10 min into the recovery period.

Exercise induced increments in heart rate, mean arterial blood pressure (MAP), CVP and IAP; however, increasing treadmill speed caused no further significant increments in any variable (Fig. 4). The increments in CVP exceeded the increments in IAP, resulting in significant increments in post caval transmural pressure (P_{TRANS}) at all treadmill speeds (Fig. 5). During the initial recovery from exercise (the first 2 min), MAP, IAP and CVP (post 0.75 and 1.0 km h⁻¹) returned to resting levels; however, heart rate (Fig. 4) and \dot{Q}_{PC} (Fig. 5) remained elevated.

Minute ventilation increased with increasing treadmill speed due to increments in tidal volume (Fig. 6). Breathing frequency, \dot{V}_{O_2} and \dot{V}_{CO_2} increased in response to exercise but did not increase further with increments in treadmill speed. All ventilatory parameters were significantly elevated during the recovery period.

Discussion

Respiratory cycle variations in IAP, CVP and \dot{Q}_{PC} were measured in alligators. IAP increased during inspiration and decreased during expiration in a pattern similar to that previously described by Farmer and Carrier (2000a). The increases in IAP during inspiration caused an increase in CVP and a substantial decrease in \dot{Q}_{PC} to near-zero values (Fig. 1). Similar inspiratory decreases in venous blood flow have been shown in supine humans, in which femoral vein blood flow falls to zero during inspiration, indicating blood stasis or arrest (Osada et al., 2002). Increments in inspired tidal volume were inversely related to \dot{Q}_{PC} , but, interestingly, although expiration

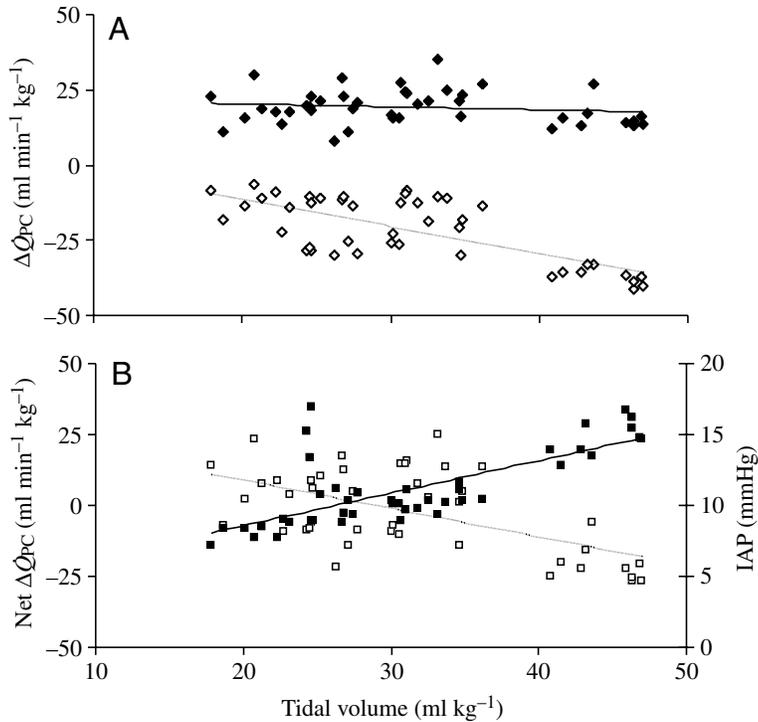
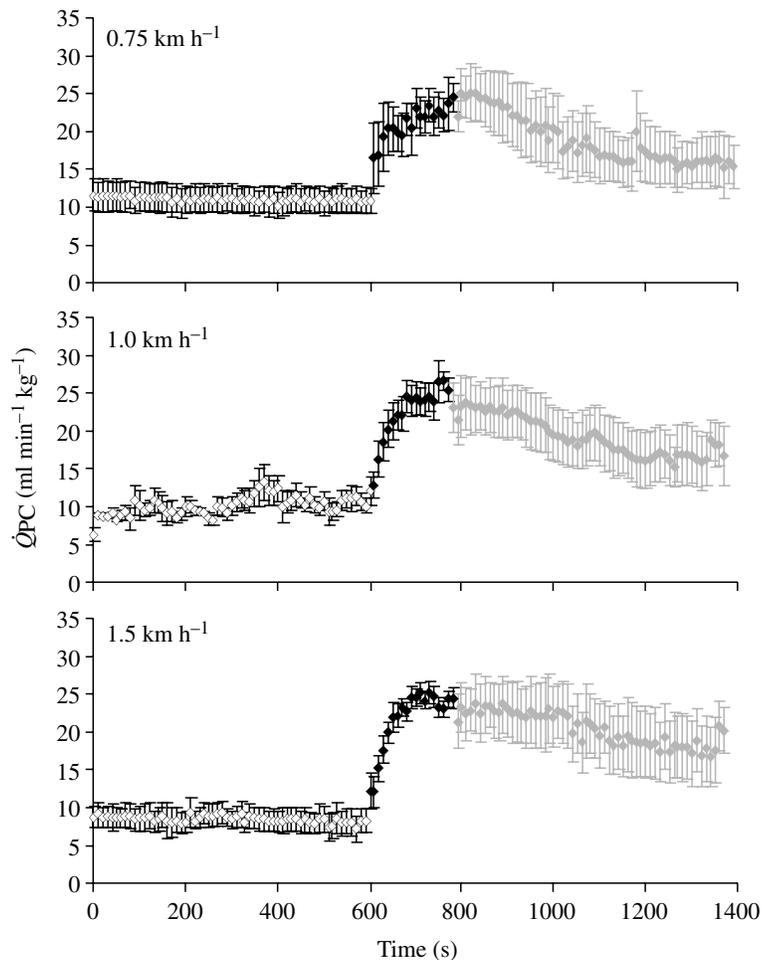


Fig. 2. Inferior vena cava flow (\dot{Q}_{PC}) and intra-abdominal pressure (IAP) vary during each breathing cycle. (A) Increasing inspired tidal volumes (open diamonds) correlate with decreasing \dot{Q}_{PC} ($r^2=-0.71$, $P<0.0001$). Expiration (filled diamonds) causes an increase in \dot{Q}_{PC} but the magnitude of the increase does not correlate with increasing tidal volumes ($r^2=-0.15$, $P=0.33$). (B) The sum of the inspiratory and expiratory alterations results in no net change in total \dot{Q}_{PC} (open squares) during the breath cycle at low tidal volumes and a net decrease at high tidal volumes. As a result, increasing tidal volumes are correlated with a net decrease in total \dot{Q}_{PC} ($r^2=-0.57$, $P<0.0001$). IAP (filled squares) is positively correlated with increments in tidal volume ($r^2=0.72$, $P<0.0001$). Data presented are 50 consecutive breaths from the recovery period post-exercise in one representative alligator (mass 3.55 kg). \dot{Q}_{PC} was calculated as the change relative to the preceding non-ventilatory period. 1 mmHg=133.3 Pa.

increased \dot{Q}_{PC} , increments in expired tidal volume were not correlated with increments in \dot{Q}_{PC} (Fig. 2A). The net change in \dot{Q}_{PC} over the respiratory cycle was inversely related to increments in tidal volume, such that at high tidal volumes (40–50 ml kg⁻¹), the inspiratory decrease in \dot{Q}_{PC} exceeded the expiratory increase, resulting in a net decrease in total \dot{Q}_{PC} over the entire respiratory cycle (Fig. 2B). These results contrast with mammalian studies in which the large tidal volumes associated with exercise supplement venous return, a condition termed the respiratory pump (Rowland, 2001). In alligators, high tidal volumes are associated with a net decrease in venous return; thus, at high tidal volumes, the mechanical act of breathing appears to inhibit rather than aid venous return. Inhibition of venous return during each respiratory cycle may impose a limitation on cardiac output and

Fig. 3. Dynamic changes in post caval flow (\dot{Q}_{PC} ; ml min⁻¹ kg⁻¹) during treadmill exercise and during the first 10 min of recovery. \dot{Q}_{PC} increases during treadmill exercise; however, there was no correlation between treadmill speed and magnitude of the \dot{Q}_{PC} change. Maximal \dot{Q}_{PC} was achieved 20 s after the onset of exercise at 0.75 km h⁻¹, and 40 s after the onset at both 1.0 and 1.5 km h⁻¹ (paired Dunnett's test). \dot{Q}_{PC} was still significantly elevated (relative to rest) during the 10th minute after all treadmill speeds. Data presented are means \pm S.E.M. for 10 s intervals ($N=5$). Open symbols represent rest; black symbols represent exercise; grey symbols represent recovery from exercise.



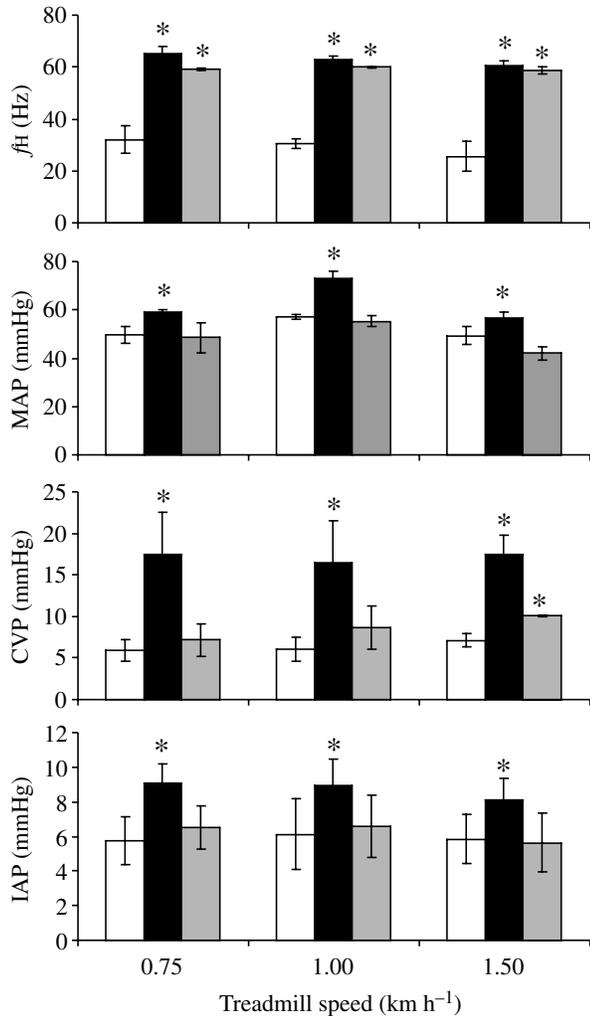


Fig. 4. Heart rate (f_H), mean arterial pressure (MAP), central venous pressure (CVP) and intra-abdominal pressure (IAP) increase during treadmill exercise. White bars indicate rest, black bars indicate exercise and grey bars indicate recovery period (first 2 min). Data presented are means \pm S.E.M. ($N=5$). * indicates a significant difference relative to rest (paired Dunnett's test, $P<0.05$). 1 mmHg=133.3 Pa.

maximal oxygen consumption during exercise when tidal volumes are elevated and the non-ventilatory period between breaths decreases.

Respiratory cycle variations in venous return, IAP and CVP have not been previously investigated in reptiles. Mammalian studies demonstrate that inspiration causes opposing alterations in intrathoracic and intra-abdominal pressure and hence have differing effects on regional venous blood flow. Although some studies on locomoting mammals demonstrate a decrease in gastric pressure during inspiration (Ainsworth et al., 1989), most studies show that the caudal translation of the diaphragm during inspiration results in compression of the viscera, increasing IAP and collapsing the inferior vena cava (Decramer et al., 1984; Guyton and Adkins, 1954; van den Berg et al., 2002; Wexler et al., 1968). Collapse of the inferior

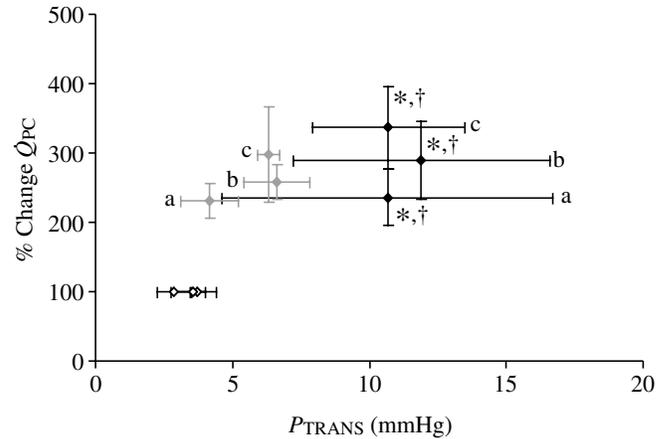


Fig. 5. The effect of exercise on inferior vena cava transmural pressure (P_{TRANS}) and % change in inferior vena cava blood flow relative to rest (\dot{Q}_{PC}). Open symbols represent rest; black symbols represent exercise; grey symbols represent recovery from exercise. Data presented are means \pm S.E.M. ($N=5$) for each treadmill speed (a, b and c represent 0.75, 1.0 and 1.5 km h⁻¹, respectively). All exercise and recovery values for \dot{Q}_{PC} (*) and P_{TRANS} (†) were significantly different relative to rest (paired Dunnett's test, $P<0.05$).

vena cava during inspiration decreases venous return from the lower extremities and is associated with a decrease in splanchnic blood flow (Abel and Waldhausen, 1969; Rabinovici and Navot, 1980; Willeput et al., 1984). Left ventricular stroke volume can also be reduced during inspiration in mammals (Charlier et al., 1974; Hoffman et al., 1965; Ruskin et al., 1973; Schrijen et al., 1975) due to a fall in effective ejection pressure of the left ventricle (Olsen et al., 1985). Contraction of the abdominal muscles during expiration aids venous return in mammals (Abel and Waldhausen, 1969; Youmans et al., 1963). In contrast to the inspiratory increase in IAP, intrathoracic and right atrial pressures decrease during inspiration in mammals, increasing blood flow in the veins located near the thorax, such as the jugular and hepatic veins and the superior vena cava (Abu-Yousef, 1992; Brecher and Hubay, 1955; Brecher and Mixter, 1953; Mixter, 1953; Moreno et al., 1967; Osada et al., 2002; Takata et al., 1992; Teichgraber et al., 1997; Willeput et al., 1984).

Venous return is proportional to the ratio of the pressure gradient between CVP and right atrial pressure. A decrease in right atrial pressure during inspiration increases the pressure gradient between the central venous circuit and the right atria, promoting venous return. In addition, venous return is inversely related to venous resistance. In collapsible vessels, such as the reptilian post caval vein, vessel cross-sectional area is largely determined by P_{TRANS} (Katz et al., 1969; Kresh and Noordergraaf, 1972; Moreno et al., 1970). When IAP exceeds CVP, P_{TRANS} becomes negative, resulting in venous vessel collapse and an increase in the viscous resistance to blood flow (Badeer and Hicks, 1992). Thus, increments in IAP during inspiration decrease venous return. The variable effects of inspiration on venous return are the result of the integration of

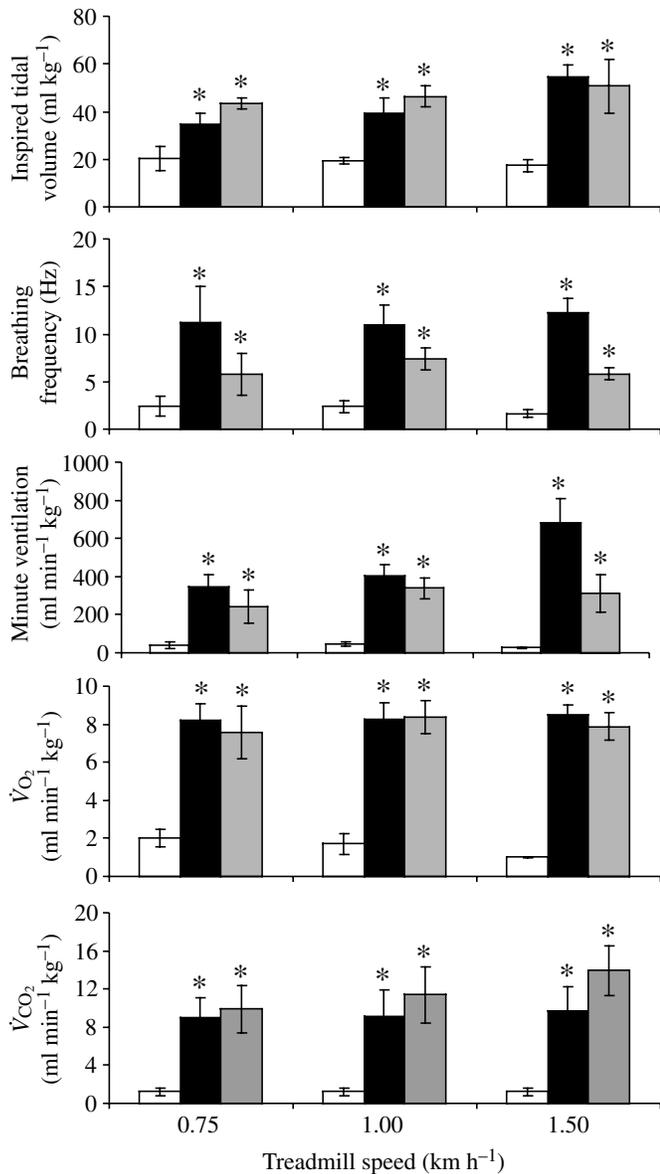


Fig. 6. Inspired tidal volume, breathing frequency, minute ventilation, rate of oxygen consumption (\dot{V}_{O_2}) and rate of carbon dioxide production (\dot{V}_{CO_2}) increase in response to treadmill exercise. White bars indicate rest, black bars indicate exercise and grey bars indicate recovery period (first 2 min). Increases in both minute ventilation and inspired tidal volume correlate with increases in treadmill speed ($r^2=0.49$, $P=0.033$; $r^2=0.47$, $P=0.041$, respectively); however, increases in breathing frequency, \dot{V}_{O_2} and \dot{V}_{CO_2} did not correlate with increases in treadmill speed ($r^2=0.012$, $P=0.78$; $r^2=0.011$, $P=0.78$, $r^2=0.174$, $P=0.26$, respectively). Data presented are means \pm S.E.M. ($N=5$). * indicates a significant difference relative to rest (paired Dunnett's test, $P<0.05$).

the effects of intrathoracic pressure and IAP on venous blood flow.

Unlike mammals, reptiles do not possess a muscular diaphragm. Crocodylians do possess a sheath of connective tissue that connects the liver to the body wall and creates separate thoracic and abdominal cavities (Grigg and Gans,

1993a; Hughes, 1973). Crocodylians are aspiration breathers, creating negative pressures within the thoracic cavity to inflate the lungs. At the same time, caudal translation of the visceral organs causes an increase in IAP. Thus, in alligators, a pressure gradient is generated between the thoracic and abdominal cavities, similar to that created during ventilation in mammals. Negative intrathoracic pressures act to decrease right atrial pressures and increase the pressure gradient, driving venous return, while increases in IAP act to compress the abdominal veins (if $IAP > CVP$). Thus, the effects of inspiration on venous return are complex and are the result of interactions between intrathoracic pressure, IAP, right atrial pressure and CVP.

Tidal volume increased in response to exercise in alligators, and increments in treadmill speed were positively correlated with increasing tidal volume (Fig. 6). The resting tidal volumes measured in this study were equivalent to those measured in previous studies (Farmer and Carrier, 2000b; Hicks and White, 1992), although the maximum tidal volumes induced by exercise in this study (55 ml kg^{-1}) were lower than those previously presented at the same treadmill speed (85 ml kg^{-1} ; Farmer and Carrier, 2000b). The significant increments in tidal volume and minute ventilation that were measured in response to increasing treadmill speed indicate that tidal volume limitations during exercise were not present in alligators. By contrast, costal tidal volumes decrease or remain unaltered with increasing treadmill speed in *Varanus exanthematicus* (Carrier, 1987; Munns et al., 2004; Wang et al., 1997). Minute ventilation during exercise was maintained in *Varanus exanthematicus* due to the contribution of the gular pump, an accessory ventilatory mechanism that forces air from the gular region into the lungs (Munns et al., 2004; Owerkowicz et al., 1999, 2001). However, it is important to note that the use of gular pumping during exercise is not universal among varanid species. *Varanus gouldi* and *Varanus spenceri* do not use gular pumping and increase tidal volumes during exercise (Frappell et al., 2002b; Schultz et al., 1999).

Gular pumping is not present in alligators (Brainerd, 1999; Farmer and Carrier, 2000a). Some movement of air in and out of the buccal region in alligators was measured in this (Fig. 1) and other studies; however, these volumes do not contribute to gas exchange (Farmer and Carrier, 2000b; Gans and Clark, 1976; Naifeh et al., 1970a,b, 1971). In contrast to the positive correlation between treadmill speed and both minute ventilation and tidal volume, rates of oxygen consumption and carbon dioxide production did not increase with treadmill speed (Fig. 6). The low maximal rates of oxygen consumption measured during exercise in alligators are indicative of the low aerobic capacity of this species.

IAP and CVP increased during treadmill exercise in alligators; however, CVP exceeded IAP at all treadmill speeds (Fig. 4), resulting in elevations in both P_{TRANS} and \dot{Q}_{PC} (Fig. 5). By contrast, elevations in IAP in varanid lizards resulted in a reduction in P_{TRANS} below zero, causing post caval vein collapse and a 52% reduction in \dot{Q}_{PC} (Munns et al., 2004). Elevations in IAP and reductions in \dot{Q}_{PC} have also been found during exercise in iguanid lizards (Farmer and Hicks,

2000). During exercise in varanid lizards, elevated IAP resulted in the collapse of the post caval vein and a pooling of blood in the peripheral circulation. During the recovery period immediately after exercise ceased, IAP fell and blood pooling in the periphery reentered the circulation, causing an increase in \dot{Q}_{PC} and MAP above exercise values (Munns et al., 2004). Peak MAP and \dot{Q}_{PC} occurred during exercise in alligators and not during the recovery period, as was the case in varanid lizards. This, in combination with the increase in P_{TRANS} , indicates that collapse of the post caval vein did not occur during exercise in alligators.

Alligators had lower IAP at rest (5.90 ± 1.78 mmHg) compared with varanid lizards (11.09 ± 2.14 mmHg) (Munns et al., 2004). The differences in IAP between varanids and alligators at rest may be due to a number of factors, including the degree of gastrointestinal filling, functional residual capacity of lungs, and resting body posture. Interestingly, exercise induced increases in IAP of similar magnitude in varanid lizards and American alligators (up to 170% and 150% of resting value, respectively). The marked differences in the effects of IAP on \dot{Q}_{PC} were the result of the interactions of IAP, CVP and right atrial pressure. The large increases in CVP (270%) in alligators were able to maintain positive P_{TRANS} during exercise, and thus prevent the inferior vena caval collapse that limited \dot{Q}_{PC} in exercising varanid lizards. Thus, the key factor determining \dot{Q}_{PC} during exercise is not absolute IAP but rather the interaction of CVP and IAP, and the maintenance of positive P_{TRANS} . The high CVP generated during exercise in alligators may be due to a number of factors. Movement of the liver and elevated IAP during the inspiration act to impede venous return and increase CVP, especially at high tidal volumes. In addition, exercise-induced peripheral vasodilation may act to increase CVP, and the liver may provide significant resistance to the flow of blood, thus increasing the resistance to venous return and increasing CVP. While high CVP values during locomotion have been measured in this study, the mechanisms underlying this increase are outside the scope of this study.

Increments in treadmill speed did not correlate with additional increases in heart rate, CVP, IAP, MAP (Fig. 4) or \dot{Q}_{PC} (Fig. 3) in alligators. Similar results were measured in varanid lizards, in which increments in treadmill speed caused no further increase in heart rate (Gleeson et al., 1980; Munns et al., 2004; Wang et al., 1997), systemic blood flow (Wang et al., 1997), \dot{Q}_{PC} (Munns et al., 2004) or stroke volume (Frappell et al., 2002a). Thus, in contrast to the graded respiratory responses to exercise in both alligators (Fig. 6) and varanid lizards (Munns et al., 2004; Owerkowicz et al., 1999; Wang et al., 1997), the maximal cardiovascular response may be reached early in the exercise period, reflecting an 'all or nothing' response at the tested treadmill speeds. In fact, peak \dot{Q}_{PC} in alligators occurred within the first 20–40 s after the onset of exercise (Fig. 3). In contrast to the all-or-nothing cardiovascular response to exercise in reptiles, the graded responses are seen in response to feeding, with incremental

increases in heart rate, stroke volume and cardiac output occurring during the first 24–48 h after feeding (Hicks et al., 2000; Secor et al., 2000). It remains probable that the cardiovascular system in both alligators and varanid lizards may demonstrate graded responses to exercise at lower intensities and that the treadmill speeds used in this and other studies may not be low enough to elicit sub-maximal cardiovascular responses. Thus, under the experimental conditions used to date, cardiovascular responses in reptiles appear to be flexible and dependent on the type of physiological stress.

In conclusion, alligators retain the ability to ventilate their lungs during locomotion, an ability lacking in some lizards. A significant increase in IAP was associated with exercise in alligators; however, a nearly 2-fold increase in CVP maintained positive P_{TRANS} , preventing both the collapse of the post caval vein and inhibition of venous return. Thus, despite both alligators and varanid lizards experiencing lateral bending of the trunk and similar elevations in IAP during exercise, the high CVP induced in alligators prevented the collapse of abdominal veins that was apparent during exercise in varanid lizards. Hemodynamic responses to exercise were rapid with peak values attained within 20–40 s after the onset of exercise. Furthermore, hemodynamic responses to exercise were not graded, showing no correlation with increments in treadmill speed. IAP, CVP and \dot{Q}_{PC} also varied during each respiratory cycle, and data suggest that while the tonically elevated IAP during exercise does not limit venous return, phasic fluctuations in IAP may act to reduce \dot{Q}_{PC} during the respiratory cycle at high tidal volumes.

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References

- Abel, F. L. and Waldhausen, J. A. (1969). Respiratory and cardiac effects on venous return. *Am. Heart J.* **78**, 266-275.
- Abu-Yousef, M. M. (1992). Normal and respiratory variation of the hepatic and portal venous duplex Doppler waveforms with simultaneous electrocardiographic correlation. *J. Ultras. Med.* **11**, 263-268.
- Ainsworth, D., Smith, C., Eicker, S., Henderson, K. and Dempsey, J. (1989). The effects of locomotion in respiratory muscle activity in the awake dog. *Respir. Physiol.* **78**, 145-162.
- Badeer, H. and Hicks, J. (1992). Hemodynamics of vascular 'waterfall': is the analogy justified? *Respir. Physiol.* **87**, 205-221.
- Bennett, A. F. and Hicks, J. (2001). Postprandial exercise: prioritization or additivity of the metabolic responses? *J. Exp. Biol.* **204**, 2127-2132.
- Brainerd, E. (1999). New perspectives on the evolution of lung ventilation mechanisms in vertebrates. *Exp. Biol. Online* **4**, 11-28.

- Brecher, G. A. and Hubay, C. A.** (1955). Pulmonary blood flow and venous return during spontaneous respiration. *Circ. Res.* **3**, 210-214.
- Brecher, G. A. and Mixer, G. J.** (1953). Effects of respiratory movements on superior vena cava flow under normal and abnormal conditions. *Am. J. Physiol.* **172**, 457-467.
- Carrier, D. R.** (1987). The evolution of locomotor stamina in tetrapods: circumventing a mechanical constraint. *Paleobiology* **13**, 326-341.
- Charlier, A. A., Jaumin, P. M. and O'Pouleur, H.** (1974). Circulatory effects of deep inspirations, blocked expirations and positive pressure inflations at equal transpulmonary pressures in conscious dogs. *J. Physiol.* **241**, 589-605.
- Decramer, M., DeTroyer, A., Kelly, S., Zocchi, L. and Macklem, P. T.** (1984). Regional differences in abdominal pressure swings in dogs. *J. Appl. Physiol.* **57**, 1682-1687.
- Farley, C. T. and Ko, T. C.** (1997). Mechanics of locomotion in lizards. *J. Exp. Biol.* **200**, 2177-2188.
- Farmer, C. G. and Carrier, D. R.** (2000a). Pelvic aspiration in the American alligator (*Alligator mississippiensis*). *J. Exp. Biol.* **203**, 1679-1687.
- Farmer, C. G. and Carrier, D. R.** (2000b). Ventilation and gas exchange during treadmill locomotion in the American alligator (*Alligator mississippiensis*). *J. Exp. Biol.* **203**, 1671-1678.
- Farmer, C. G. and Hicks, J. W.** (2000). Circulatory impairment induced by exercise in the lizard *Iguana iguana*. *J. Exp. Biol.* **203**, 2691-2697.
- Frappell, P., Schultz, T. and Christian, K.** (2002a). Oxygen transfer during aerobic exercise in a varanid lizard *Varanus mertensi* is limited by the circulation. *J. Exp. Biol.* **205**, 2725-2736.
- Frappell, P., Schultz, T. and Christian, K.** (2002b). The respiratory system in varanid lizards: determinants of O₂ transfer. *Comp. Biochem. Physiol.* **133A**, 239-258.
- Gans, C. and Clark, B.** (1976). Studies on ventilation of *Caiman crocodylus* (Crocodilia: Reptilia). *Respir. Physiol.* **26**, 285-301.
- Gleeson, T., Mitchell, G. and Bennett, A. F.** (1980). Cardiovascular responses to graded activity in the lizards *Varanus* and *Iguana*. *Am. J. Physiol.* **239**, R174-R179.
- Grigg, G. and Gans, C.** (1993a). Morphology and physiology of the crocodylia. In *Fauna of Australia*, vol. 2A (ed. C. J. Glasby, G. J. B. Ross and P. L. Beesley), pp. 326-336. Canberra: Australian Government Publishing Service.
- Guyton, A. C. and Adkins, L. H.** (1954). Quantitative aspects of the collapse factor in relation to venous return. *Am. J. Physiol.* **177**, 523-527.
- Hartzler, L. K., Munns, S. L. and Hicks, J. W.** (2004). Contribution of the hepatic piston to ventilation in the American alligator. *FASEB J.* **18**, Abstr. 238.4.
- Hicks, J. W. and White, F. N.** (1992). Pulmonary gas exchange during intermittent ventilation in the American alligator. *Respir. Physiol.* **88**, 23-36.
- Hicks, J., Wang, T. and Bennett, A. F.** (2000). Patterns of cardiovascular and ventilatory response to elevated metabolic states in the lizard *Varanus exanthematicus*. *J. Exp. Biol.* **203**, 2437-2445.
- Hoffman, J. I. E., Gutz, A., Charlier, A. A. and Wicken, D. E. L.** (1965). Stroke volume in conscious dogs; effects of respiration, posture, and vascular occlusion. *J. Appl. Physiol.* **20**, 865-877.
- Hughes, G. M.** (1973). *The Vertebrate Lung*. London: Oxford University Press.
- Katz, A., Chen, Y. and Moreno, A.** (1969). Flow through a collapsible tube. Experimental analysis and mathematical model. *Biophys. J.* **9**, 1261-1279.
- Kresh, E. and Noordergraaf, A.** (1972). Cross-sectional shape of collapsible tubes. *Biophys. J.* **12**, 274-294.
- Mixer, G. J.** (1953). Respiratory augmentation of inferior vena caval flow demonstrated by a low-resistance phasic flowmeter. *Am. J. Physiol.* **172**, 446-456.
- Moreno, A., Burchell, A. R., Van der Woude, R. and Burke, J. H.** (1967). Respiratory regulation of splanchnic and systemic venous return. *Am. J. Physiol.* **213**, 455-465.
- Moreno, A., Katz, A., Gold, L. and Reddy, R.** (1970). Mechanics of distension of dog veins and other very thin-walled tubular structures. *Circ. Res.* **27**, 1067-1080.
- Munns, S. L., Hartzler, L. K., Bennett, A. F. and Hicks, J. W.** (2004). Elevated intra-abdominal pressure limits venous return during exercise in *Varanus exanthematicus*. *J. Exp. Biol.* **207**, 4111-4120.
- Naifeh, K. H., Huggins, S. E. and Hoff, H. E.** (1970a). The nature of the ventilatory period in crocodylian respiration. *Respir. Physiol.* **10**, 338-348.
- Naifeh, K. H., Huggins, H. E., Hoff, H. E., Hugg, T. W. and Norton, R. E.** (1970b). Respiratory pattern in crocodylian reptiles. *Respir. Physiol.* **9**, 31-42.
- Naifeh, K. H., Huggins, S. E. and Hoff, H. E.** (1971). The nature of the nonventilatory period in crocodylian respiration. *Respir. Physiol.* **11**, 178-185.
- Olsen, C. O., Tyson, M. S., Mier, G. W., Davies, J. W. and Rankin, R.** (1985). Diminished stroke volume during inspiration: a reverse thoracic pump. *Circulation* **72**, 668-679.
- Osada, T., Katsumura, T., Hamaoka, T., Murase, N., Naka, M. and Shimomitsu, T.** (2002). Quantitative effects of respiration on venous return during single knee extension-flexion. *Int. J. Sports. Med.* **23**, 183-190.
- Owerkowicz, T., Farmer, C. G., Hicks, J. W. and Brainerd, E. L.** (1999). Contribution of gular pumping to lung ventilation in monitor lizards. *Science* **284**, 1661-1663.
- Owerkowicz, T., Brainerd, E. and Carrier, D.** (2001). Electromyographic pattern of the gular pump in monitor lizards. *Bull. Mus. Comp. Zool.* **156**, 237-248.
- Rabinovici, N. and Navot, N.** (1980). The relationship between respiration, pressure and flow distribution in the vena cava and portal and hepatic veins. *Surg. Gynecol. Obstet.* **151**, 753-763.
- Reilly, S. M. and Blob, R. W.** (2003). Motor control of locomotor hindlimb posture in the American alligator (*Alligator mississippiensis*). *J. Exp. Biol.* **206**, 4327-4340.
- Reilly, S. M. and Elias, J. A.** (1998). Locomotion in Alligator mississippiensis: Kinematic effects of speed and posture and their relevance to the sprawling-to-erect paradigm. *J. Exp. Biol.* **201**, 2559-2574.
- Rowland, T.** (2001). The circulatory response to exercise: role of the peripheral pump. *Int. J. Sports. Med.* **22**, 558-565.
- Ruskin, J., Bache, R. J., Rembert, J. C. and Greenfield, J. C.** (1973). Pressure-flow studies in man: Effect of respiration on left ventricular volume. *Circ. Res.* **48**, 79-85.
- Schaffner, F.** (1998). The Liver. In *Biology of the Reptilia*, vol. 19 (ed. C. Gans and A. Gaunt), pp. 485-531. Ithaca, NY: Society for the Study of Amphibians and Reptiles.
- Schrijen, F., Ehrlich, W. and Permutt, S.** (1975). Cardiovascular changes in conscious dogs during spontaneous deep breaths. *Pflügers Arch.* **355**, 205-215.
- Schultz, T., Christain, K. and Frappell, P.** (1999). Do lizards breathe through their mouth while running? *Exp. Biol. Online* **4**, 39-46.
- Secor, S., Hicks, J. and Bennett, A.** (2000). Ventilatory and cardiovascular responses of a python (*Python molurus*) to exercise and digestion. *J. Exp. Biol.* **203**, 2447-2454.
- Takata, M., Beloucif, S., Shimada, M. and Robotham, J. L.** (1992). Superior and inferior vena caval flows during respiration: pathogenesis of Kussmaul's sign. *Am. J. Physiol.* **262**, H763-H770.
- Teichgraber, U., Gebel, M., Benter, T. and Manns, M.** (1997). Effects of respiration, exercise, and food intake on hepatic vein circulation. *J. Ultras. Med.* **16**, 549-554.
- van den Berg, P. C. M., Jansen, J. R. C. and Pinsky, M. R.** (2002). Effect of positive pressure on venous return in volume-loaded cardiac surgical patients. *J. Appl. Physiol.* **92**, 1223-1231.
- Wang, T., Carrier, D. and Hicks, J.** (1997). Ventilation and gas exchange in lizards during treadmill exercise. *J. Exp. Biol.* **200**, 2629-2639.
- Wexler, L., Bergel, D. H., Gabe, I. T., Makin, G. S. and Mills, C. J.** (1968). Velocity of blood flow in normal human vena cavae. *Circ. Res.* **23**, 349-359.
- Willeput, R., Rondeux, C. and De Troyer, A.** (1984). Breathing affects venous return from legs in humans. *J. Appl. Physiol.* **57**, 971-976.
- Youmans, W. B., Murphy, Q. R., Turner, J. K., Davis, L. D., Briggs, D. I. and Hoye, A. S.** (1963). Activity of abdominal muscles elicited from the circulatory system. *Am. J. Phys. Med.* **42**, 1-70.