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The cost of ventilation in birds measured via unidirectional artificial ventilation

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ABSTRACT

The highly derived mechanism birds use to ventilate their lungs relies on dorsoventral excursions of their heavily muscled sternum and abdominal viscera. Our expectation of the level of mechanical work involved in this mechanism led us to hypothesize that the metabolic cost of breathing is higher in birds than in other tetrapods. To test this theory, we used unidirectional artificial ventilation (UDV) to stop normal ventilatory movements in guinea fowl (*Numida meleagris* L.) at rest and during treadmill locomotion at three speeds. Oxygen consumption was measured during normal breathing and UDV, and the difference was used to approximate the cost of ventilation. Contrary to our prediction, metabolism increased when ventilatory movements ceased during UDV at rest. Although we do not understand why this occurred we suspect that UDV induced a homeostatic mechanism to counteract the loss of carbon dioxide. Nevertheless, across all running speeds, metabolism decreased significantly with UDV, indicating a minimum cost of ventilation during running of $1.43 \pm 0.62\%$ of total running metabolism or 0.48 ± 0.21 mL O_2 (L ventilated)$^{-1}$. These results suggest that the metabolic cost of ventilation is low in birds and that it is within the range of costs reported previously for other amniotes.

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1. Introduction

The cost of resting ventilation is generally thought to be a few percent of total resting metabolism in mammals and non-avian sauropsids (Roussos and Zakynthinos, 1995; Skovgaard and Wang, 2007). During exercise, the cost of ventilation has been shown to increase with increasing ventilation rate in mammals and has been measured to be as much as 18% of running metabolism or 1.5 to 12 mL O_2 (L ventilated)$^{-1}$ (Marconi et al., 1982; Art et al., 1990; Roussos and Zakynthinos, 1995; Dempsey et al., 1996; Marks et al., 2005; Vella et al., 2006).

The energetic cost of lung ventilation may be relatively high in birds because ventilation is driven primarily by dorsoventral movements of the large sternum. Supported by the sternum are the massive flight muscles (which average 17% body mass across many species; Greenewalt, 1962) and the abdominal viscera (King, 1966). In guinea fowl, the mass of all these structures is approximately 25% of body mass. Thus the metabolic cost of breathing must include the mechanical energy used to lower and to raise this massive complex. In a study designed to measure energy expenditure of individual limb muscles during locomotion in guinea fowl, Ellerby et al. (2005) estimated the cost of ventilation as the increase in total blood flow with exercise minus the increase in blood flow to locomotor and heart muscles. They estimated the cost of ventilation to be 2% of whole body metabolism, but this study did not measure the cost of ventilation directly, and to our knowledge no other study has measured the cost of ventilation in birds. This study tests the hypothesis that the cost of ventilation is higher in birds than in other amniotes.

The cost of ventilation has been estimated in swimming fishes by measuring the drop in metabolism when fish changed their mode of breathing from brachial to ram ventilation; this change in metabolism was assumed to represent the cost of active brachial ventilation (Freadman, 1981; Steffensen, 1985). The unique structure of the avian lung–air sac system allows us to use similar methods to measure the metabolic cost of breathing at rest and during running. Birds can be unidirectionally artificially ventilated (UDV) by cannulating the caudal air sacs and pumping air into the respiratory system at a steady flow rate (first proposed by Burger and Lorenz, 1960). If the rate of airflow is increased sufficiently, gas exchange will lower arterial levels of carbon dioxide, reducing the drive to breathe until ventilatory movements cease, while gas exchange continues to take place. We assumed that non-ventilatory oxygen consumption remains constant and that the difference in oxygen uptake measured in birds with and without ventilatory movements is equivalent to the cost of ventilation.

This technique has been used in several studies of the control of ventilation in resting and running birds (review by Kiley and Fedde, 1983; Scheid and Piiper, 1986; Estavillo et al., 1990; Adamson and Solomon, 1993). UDV has also been used to measure the cost of ventilation in a species of turtle (Kinney and White, 1977). The

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purpose of this study was to measure the cost of ventilation at rest and during running in guinea fowl and to determine how the cost of ventilation compares with that of other vertebrates.

2. Materials and methods

Guinea fowl (*Numida meleagris* L.) were obtained from a local breeder. All animal procedures conformed to the requirements of the Institutional Animal Care and Use Committee of the University of Utah (Protocol Number 05-08010). Four birds (1.45 ± 0.07 kg) were trained to run on a treadmill for over 10 min at each of three speeds (1.6, 1.9 and 2.4 m s^{-1}) for 6 to 8 weeks before the start of the experiment. During this time, birds were also trained to wear a mask and to become accustomed to airflow through this mask.

Following training, guinea fowl were induced for surgery by using a mask ventilated with 5% isoflurane in room air, then intubated and maintained on a ventilator with 1–2% isoflurane. Feathers at the surgical sites were plucked. Surgery was performed with aseptic techniques. A Tygon tube (S-50-HL, 1/8 in. i.d., 3/16 in. o.d., Saint-Gobain Performance Plastics, Beaverton, MI, USA) was implanted into each abdominal air sac via an incision ventral to the pubis bone and anchored to this bone with suture to allow for unidirectional artificial ventilation (UDV) flow into these air sacs and out the mouth and nares.

After a 2-day recovery period, oxygen consumption was measured in awake birds as shown in Fig. 1. Masks were custom made from Plexiglas to fit each bird. Two Clean-Bor tubes (10 mm i.d., VacuMed, Ventura, CA, USA) provided inlet and outlet bias airflows supplied by two aquarium pumps (Profile 9500 Aquarium Air Pump, PETCO Animal Supplies, Inc., San Diego, CA 92121). The abdominal air sac cannulae were connected to a separate aquarium pump to provide UDV flow. Water and carbon dioxide were removed, with Drierite (W.A. Hammond Drierite Co. Ltd., Xenia, OH, USA) and Sodasorb (W.R. Grace & Co., Conn, Cambridge, MA, USA), respectively, from mask and UDV air before measuring flow rates with Smart-Trak® Model 100 Mass Flow meters (Sierra Instruments, Monterey, CA, USA). UDV air was then humidified before entering the bird's air sacs by passing it through a heated beaker of water. Ventilation and UDV flow out the nares and mouth were recorded via a heated pneumotachograph (model 8300, Hans Rudolph, Kansas City, MO, USA) downstream from the mask. A sub-sample of air downstream from the pneumotachograph was taken with an R1 Flow Control unit (AEI Technologies, Inc., Pittsburgh, PA, USA). Water was removed from a sub-sample of that air before measuring the carbon dioxide content with a carbon dioxide analyzer (CD-3A, AEI Technologies, Inc.), and carbon dioxide and water were removed before measuring the oxygen content with an oxygen analyzer (S3-A/1, AEI Technologies, Inc.). The flow through both analyzers was controlled by a second R1 Flow Control unit. The bird was also outfitted with an accelerometer (Microtron, 7290A-10, Endevco Corp., San Juan Capistrano, CA, USA) to measure its movements at rest and during running. Ventilation, mask and UDV flows, oxygen and carbon dioxide content, and acceleration data were collected by using LabView 7 (CB-68-LP external Data Acquisition Board, PCI-6034E internal board, National Instruments Corp., Austin, TX, USA) running on a PowerMac desktop computer (Apple Inc, Cupertino, CA, USA).

Birds were placed on a box-covered treadmill for data collection. All sides of this box were covered by an opaque cloth throughout resting trials. During running trials, a Plexiglas side of the box was uncovered to allow video recording. A fan placed at the open back of the treadmill cooled the bird during running trials, and a mirror was placed at the closed front of the treadmill to improve running performance.

For UDV trials, the UDV rate was chosen by incrementally increasing the flow a few deciliters at a time until no breathing was evident on the pneumotachograph trace. UDV was then decreased slightly and the process was repeated to find the approximate minimum UDV rate required to stop ventilation. In subsequent UDV trials on the same day, UDV was turned on at the previously used UDV rate and then adjusted slightly if necessary. Breathing restarted within 30 s of turning off UDV in all trials; however, in most resting trials breathing restarted within 15 s, and in all running trials breathing restarted in 6 s or less.

Data were collected for 5 min trials while the bird was breathing naturally, followed by 5 min while the bird was not breathing because of UDV. In preliminary trials, when UDV was performed for longer

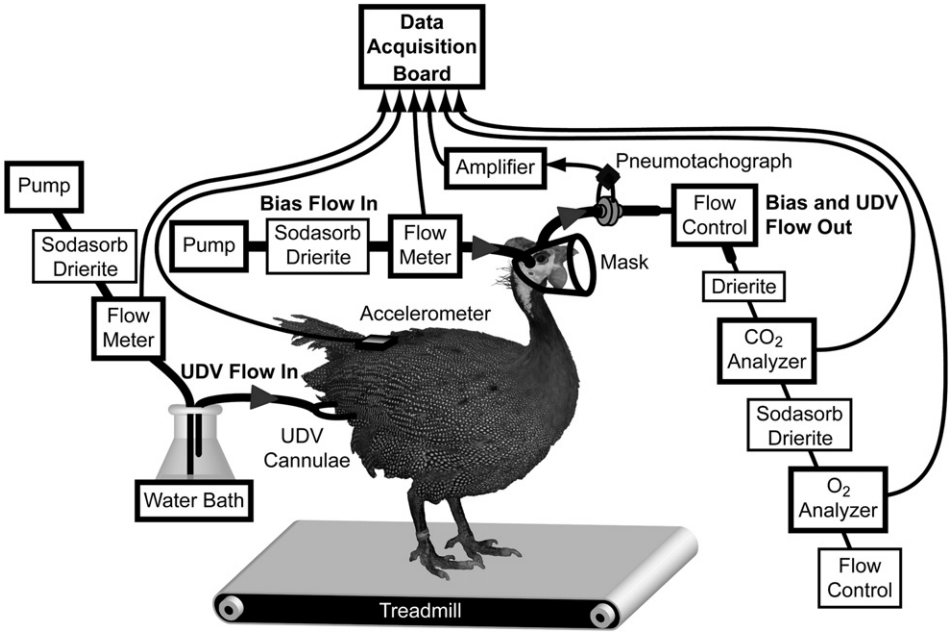

Fig. 1. Experimental setup used to measure respiration and metabolism. Bold boxes represent equipment, light boxes absorbents. Pumps are aquarium pumps, which pushed air. Sodasorb absorbs CO_2 and Drierite absorbs water. Flow Meter and Pneumotachograph measured flow rates. Flow Control pulled air into gas analyzers. The Water Bath heated and humidified air. UDV Cannulae are tubes surgically implanted in the bird's abdominal air sacs. The Data Acquisition Board digitized signals from the Flow Meters, Accelerometer, Pneumotachograph, CO_2 Analyzer, and O_2 Analyzer and then passed them to a computer.

Table 1
Mean ventilation and metabolic rates pre- and post-surgery.

	$\dot{V}_E$ (mL min^{-1}kg^{-1})	$\dot{V}_{O_2}$ (mL O_2 min^{-1}kg^{-1})	$\dot{V}_{CO_2}$ (mL CO_2 min^{-1}kg^{-1})	RER
Resting				
Pre-surgery	0.36 ± 0.03	12.4 ± 1.1	9.7 ± 1.3	0.77 ± 0.05
Post-surgery	0.32 ± 0.04	13.1 ± 0.6	10.4 ± 0.9	0.80 ± 0.03
Running 1.6 m s^{-1}				
Pre-surgery	1.91 ± 0.20	58.3 ± 4.3	50.3 ± 6.5	0.85 ± 0.06
Post-surgery	1.56 ± 0.16	57.0 ± 2.9	48.2 ± 4.1	0.84 ± 0.04
Running 1.9 m s^{-1}				
Pre-surgery	2.09 ± 0.23	63.9 ± 4.7	52.1 ± 6.5	0.80 ± 0.05
Post-surgery	1.76 ± 0.11	64.6 ± 1.8	52.9 ± 3.8	0.82 ± 0.05
Running 2.4 m s^{-1}				
Pre-surgery	2.94 ± 0.34	75.1 ± 4.4	65.0 ± 7.3	0.86 ± 0.06
Post-surgery	2.52 ± 0.31	78.8 ± 2.9	67.6 ± 4.6	0.86 ± 0.05

Reported values are means ± S.E. $N = 4$. *Abbreviations*: $\dot{V}_E$, minute ventilation (rate of expiration, BTPS); $\dot{V}_{O_2}$, O_2 consumption (STP); $\dot{V}_{CO_2}$, CO_2 loss (STP); RER, respiratory exchange ratio ($\dot{V}_{CO_2} \cdot \dot{V}_{O_2}^{-1}$). $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and RER did not change post surgery ($p > 0.6$), while $\dot{V}_E$ decreased ($p = 0.019$).

periods of time, the oxygen consumption during minutes 3 through 5 of UDV was not significantly different from that during minutes 5 through 10. In preliminary trials, birds had symptoms of metabolic distress (gout) assumed to be due to blowing off too much CO_2 during UDV. Therefore, each UDV trial was followed by 5 min of the bird breathing hypercapnic air (2% CO_2) in order to roughly replenish the bird's internal CO_2 levels. Oxygen consumption was not measured during the hypercapnic trials. These three trials, breathing, UDV and supplemental CO_2, constituted one 'round' of data collection, yielding one cost of ventilation data point. Because only four to seven rounds could be collected in a single day, data were collected on multiple days for each bird. Measurements of room O_2 and CO_2 levels and voltages at zero flow for the flow meters and pneumotachograph were taken before and after each data collection round. The same procedure was used in resting and running birds.

The posture and activity level of guinea fowl were monitored during recordings, and rounds in which animals behaved dissimilarly during paired breathing and UDV trials were excluded from the analysis. Portions of each trial with intermittent movement (i.e. shaking of the head during rest or inconsistent running strides) were also excluded. Only the last three min of each breathing and UDV trial were analyzed. Then the 2-min period with the highest oxygen content (lowest consumption) was assessed for oxygen extraction, carbon dioxide loss, minute ventilation (BTPS), and tidal volume. Oxygen extraction ($\dot{V}_{O_2}$) and carbon dioxide loss ($\dot{V}_{CO_2}$) were calculated using the equations of Withers (2001):

$$\dot{V}_{O_2} = \dot{Q}_{in} \cdot \left(\frac{F_{inO_2} - F_{exO_2}}{1 - F_{exO_2}} \right), \quad \dot{V}_{CO_2} = \dot{Q}_{in} \cdot \left(\frac{F_{exCO_2} \cdot (1 - F_{inO_2})}{(1 - F_{exO_2}) \cdot (1 - F_{exCO_2})} \right)$$

where $\dot{Q}_{in}$ is the airflow rate into the mask, F_{inO_2} is the inspired fractional concentration of O_2 (0.2095 for room air), and F_{exO_2} and F_{exCO_2} are the fractional concentrations of O_2 and CO_2 leaving the mask. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ (STP) were compared between paired breathing and UDV trials and respiratory exchange ratios (RER, $\dot{V}_{CO_2} \cdot \dot{V}_{O_2}^{-1}$) were calculated for all trials. Changes in $\dot{V}_{O_2}$ and minute ventilation ($\dot{V}_E$) were used to calculate the oxygen consumed per unit ventilation ($\Delta\dot{V}_{O_2} \cdot \Delta\dot{V}_E^{-1}$, mL of O_2 (L ventilated)$^{-1}$).

Trials in which apnea was not evident within 30 s of UDV-on at rest and within 100 s of UDV-on during running were excluded from the analysis. A stable running speed was achieved within 10 s of when the treadmill was turned on in all running trials. In approximately half of running UDV trials, small breath-like fluctuations (unrelated to movement artifact) were evident in the pneumotachograph trace, but the minute ventilation of these fluctuations was very small and ranged from 4.5 to 15% of the paired breathing running $\dot{V}_E$ in all trials used in the analysis. Any ventilation that occurred during UDV was taken into account when calculating $\Delta\dot{V}_{O_2} \cdot \Delta\dot{V}_E^{-1}$. Analysis of the difference between treatments was ascertained using analysis of covariance (ANCOVA) with bird identity as a random effect, and further comparison of treatments were calculated using Wilcoxon Sign-Rank tests for paired comparisons, least squares regression analysis (JMP 7, SAS Institute, Cary, NC, USA), Friedman's test or Kruskal–Wallis test (XLSTAT 2009.1.01, Addinsoft, New York, NY, USA). As resting and running rounds yielded distinct results, these data were analyzed separately.

3. Results

Surgical implantation of tubes in the abdominal air sacs did not affect rates of oxygen consumption. Oxygen consumption ($\dot{V}_{O_2}$), carbon dioxide loss ($\dot{V}_{CO_2}$), and respiratory exchange ratios (RER, $\dot{V}_{CO_2} \cdot \dot{V}_{O_2}^{-1}$) during normal breathing (at rest and during running) were not significantly different from pre-surgery values (Wilcoxon Sign-Rank: $p > 0.6$ for each variable; Table 1). However, ventilation rates were significantly lower after surgery (Wilcoxon Sign-Rank: $p = 0.019$) due to a significant decrease in tidal volume (Wilcoxon Sign-Rank: $p = 0.0052$; but no change in breath frequency, $p > 0.15$).

Whole body oxygen consumption rates while breathing normally were similar to those previously reported for guinea fowl. Our measurements are slightly lower than $\dot{V}_{O_2}$ values for guinea fowl at rest (13 to 30% less depending on posture; Ellerby et al., 2003; Ellerby and Marsh, 2006) and during running (6 to 10% less; Ellerby et al., 2003). The fastest running speed used in this experiment (2.4 m s^{-1}) approaches but is below the minimum running speed known to elicit maximal oxygen consumption in guinea fowl (2.8 m s^{-1}; Ellerby et al., 2003). Mean $\dot{V}_{O_2}$ at the highest running speed of this experiment is 78% of the reported $\dot{V}_{O_2max}$ for guinea fowl.

3.1. Resting metabolism during normal breathing and artificial ventilation

Oxygen consumption was higher during UDV than during normal breathing, but not significantly so. On average the birds increased their oxygen consumption by 18.4% during UDV (%$\Delta\dot{V}_{O_2}$, Table 2). This result led us to test several possible explanations.

We hypothesized that the elevated metabolism during UDV at rest may have been the result of stress from the sensation of UDV. Thus, on alternate experimental days we administered analgesics or sedatives. Valium and Buperphorine decreased the resting metabolism of birds while breathing and not breathing and changed their behavior (birds would not run), but did not change the metabolic response to UDV. Therefore data from days when Valium and Buperphorine were administered were excluded from the analyses. Resting metabolism when Acepromazine was administered to birds (*Ace* rounds) was not different from that when no drugs were administered.

We also hypothesized that the subjects were using more energy during UDV to maintain body temperature when faced with the

Table 2
Mean ventilation and metabolic rates while breathing, and not breathing.

Speed ($m\ s^{-1}$)	Vent.	$\dot{V}_E$ ($L\ min^{-1}\ kg^{-1}$)	UDV rate ($L\ min^{-1}\ kg^{-1}$)	$\dot{V}_{O_2}$ ($mL\ O_2\ min^{-1}\ kg^{-1}$)	$\Delta\dot{V}_{O_2}$	$\dot{V}_{CO_2}$ ($mL\ CO_2\ min^{-1}\ kg^{-1}$)	$\Delta\dot{V}_{CO_2}$	$\%\Delta\dot{V}_{O_2}$	$\Delta\dot{V}_{O_2}\cdot\Delta\dot{V}_E^{-1}$ ($mL\ O_2\ L^{-1}$)	$\%\Delta\dot{V}_{CO_2}$
Rest	Normal	0.32 ± 0.04		13.1 ± 0.6	−2.3 ± 0.9	10.4 ± 0.9	−5.1 ± 0.7	−18.4 ± 6.7	−7.55 ± 2.79	−49.0 ± 5.7
	UDV		0.39 ± 0.03	15.4 ± 1.2		15.5 ± 1.4				
1.6	Normal	1.56 ± 0.16		57.0 ± 2.9	−0.1 ± 0.8	48.2 ± 4.1	−1.4 ± 1.1	−0.28 ± 1.35	−0.14 ± 0.53	−4.32 ± 2.82
	UDV		1.04 ± 0.14	57.1 ± 2.8		49.6 ± 3.5				
1.9	Normal	1.76 ± 0.11		64.6 ± 1.8	0.6 ± 0.9	52.9 ± 3.8	−1.1 ± 1.8	1.00 ± 1.42	0.39 ± 0.54	−2.77 ± 4.05
	UDV		1.20 ± 0.15	63.7 ± 2.1		53.9 ± 3.2				
2.4	Normal	2.52 ± 0.31		78.8 ± 2.9	3.0 ± 0.7	67.6 ± 4.6	3.6 ± 1.5	3.58 ± 0.87	1.14 ± 0.37	5.25 ± 2.58
	UDV		1.41 ± 0.22	75.9 ± 2.5		64.4 ± 4.8				

$N = 4$. Reported values are means ± S.E. *Abbreviations*: Normal, breathing; UDV, not breathing due to unidirectional artificial ventilation; $\dot{V}_E$, minute ventilation (rate of expiration, BTPS); UDV rate, artificial ventilation flow rate (BTPS); $\dot{V}_{O_2}$, O_2 consumption (STP); $\Delta\dot{V}_{O_2}$, normal $\dot{V}_{O_2}$ minus UDV $\dot{V}_{O_2}$; $\dot{V}_{CO_2}$, CO_2 loss (STP), $\Delta\dot{V}_{CO_2}$, normal $\dot{V}_{CO_2}$ minus UDV $\dot{V}_{CO_2}$; $\%\Delta\dot{V}_{O_2}$, $\Delta\dot{V}_{O_2}$, as a percent of normal $\dot{V}_{O_2}$; $\Delta\dot{V}_{O_2}\cdot\dot{V}_E^{-1}$, unit cost of ventilation, $\Delta\dot{V}_{O_2}$ divided by change in ventilation; $\%\Delta\dot{V}_{CO_2}$, $\Delta\dot{V}_{CO_2}$, as a percent of normal $\dot{V}_{CO_2}$. UDV was lower than $\dot{V}_E$ during running at all speeds ($p = 0.0005$). During running, $\dot{V}_{O_2}$ decreased during UDV ($p = 0.0176$). $\%\Delta\dot{V}_{CO_2}$ increased with running speed ($p = 0.0216$).

cooling effect of the room temperature UDV flow. But heating the UDV air to body temperature as it entered the bird did not decrease oxygen consumption during UDV, and even appeared to increase metabolism during UDV in some rounds.

We also suspected that the hypercapnic air trial may have suppressed metabolism during the subsequent normal breathing trial. However, we found that withholding the hypercapnic air trial did not change metabolism during the normal breathing trials. Also, oxygen consumption during the first breathing trial of the day (which was not preceded by breathing hypercapnic air) was not different from that of the last breathing trail of the day (which was preceded by exposure to hypercapnic air; Wilcoxon Sign-Rank: $p > 0.17$).

Carbon dioxide loss tended to be higher during UDV than during breathing (Table 2). Thus, we hypothesized that the loss of CO_2 necessary to stop ventilation by UDV induces an unknown homeostatic mechanism to regulate pH. If this is true, the increase in metabolism during UDV should be augmented by higher UDV flow rates, which would cause greater CO_2 loss. To test this possibility, UDV flow rates were increased to a level 1.5 or 2 times that necessary to stop ventilation (as measured that day) at rest. Oxygen consumption increased in trials with these high UDV flow rates (standard least squares regression: $p = 0.0173$; Fig. 2).

Oxygen consumption while breathing and not breathing (UDV) were not different in control rounds, in *heat* rounds (bird temperature UDV air), or in *Ace* rounds (ANCOVA: $p = 0.99$). Therefore control, *heat*, and *Ace* data were pooled for each bird in subsequent analyses.

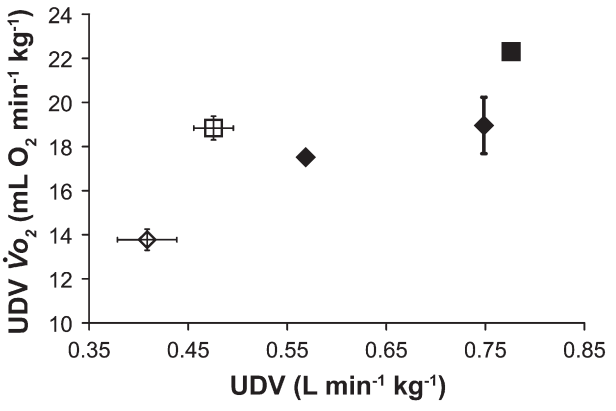

Fig. 2. Resting oxygen consumption during artificial ventilation at different artificial ventilation rates. UDV $\dot{V}_{O_2}$ is oxygen consumption during unidirectional artificial ventilation (UDV). Open symbols show minimal UDV rates required for the bird to stop breathing, while closed symbols show elevated UDV rates for two birds (represented by diamonds or squares). Error bars show S.E.; points without error bars are the means of two trials. Increasing the UDV rate above the minimum required to stop ventilation increased the oxygen consumed during artificial ventilation for both birds (standard least squares regression: $p = 0.0173$).

3.2. Running metabolism during normal breathing and artificial ventilation

In contrast to what we observed during rest, the oxygen consumption of running birds decreased significantly when ventilation ceased due to UDV (Wilcoxon Sign-Rank: $p = 0.0176$; $\Delta\dot{V}_{O_2}$, Table 2). This effect was most evident in the trials at the highest running speed, but $\Delta\dot{V}_{O_2}$ did not change significantly with speed. In contrast, carbon dioxide loss did not change significantly between breathing and UDV trials (Wilcoxon Sign-Rank: $p > 0.6$; Table 2), however the change in carbon dioxide loss ($\Delta\dot{V}_{CO_2}$) significantly increased with speed (standard least squares regression: $p = 0.017$). When compared with breathing trials, carbon dioxide loss tended to increase during UDV at the lowest speed, to not change at the medium speed, and to decrease during UDV at the highest running speed.

3.3. Cost of ventilation during running

Percent reduction in O_2 consumption while not breathing tended to increase with UDV ventilation rate and speed, but not significantly ($\%\Delta\dot{V}_{O_2}$, Table 2). The mean $\%\Delta\dot{V}_{O_2}$ for all birds across all speeds was 1.44 ± 0.81%. The unit cost of ventilation ($\Delta\dot{V}_{O_2}\cdot\dot{V}_E^{1}$) also did not change significantly with speed (Table 2). Mean $\Delta\dot{V}_{O_2}\cdot\dot{V}_E^{1}$ across all speeds was 0.46 ± 0.30 mL O_2 (L ventilated)$^{-1}$. Although the variance of $\%\Delta\dot{V}_{O_2}$ and $\Delta\dot{V}_{O_2}\cdot\dot{V}_E^{1}$ were high, across all speeds the 95% confidence intervals for each mean did not include zero.

3.4. Mismatch between ventilation and UDV rates

Parabronchial ventilation was not measured in this study, but, with certain assumptions, the relative ventilation during normal breathing and UDV may be discussed. It was assumed, conservatively, that dead space volume during normal breathing was twice the volume of the trachea (as shown by Hastings and Powell, 1986; tracheal volume calculated from the scaling equation of Hinds and Calder, 1971) and that 90% of UDV flow went through the lungs (UDV_{effect}). Using these methods, UDV_{effect} was higher than effective $\dot{V}_E$ ($\dot{V}_{E\ effect}$) at rest, although not significantly; however, during running, $\dot{V}_{E\ effect}$ was significantly greater than UDV_{effect} (Wilcoxon Sign-Rank: $p = 0.0005$; Fig. 3). Therefore, it seems that at rest UDV over-ventilated the birds, whereas during running UDV under-ventilated them relative to their normal ventilation rates.

Relative UDV rates (UDV_{effect} divided by $\dot{V}_{E\ effect}$) were calculated for each round. Relative UDV rates greater than one indicate over-ventilation during UDV, whereas rates less than one indicate under-ventilation during UDV. Relative UDV rates were significantly higher at rest (1.93 ± 0.18) than during running (0.86 ± 0.05; Kruskal–Wallis test: $p < 0.0001$). The change in oxygen consumption between

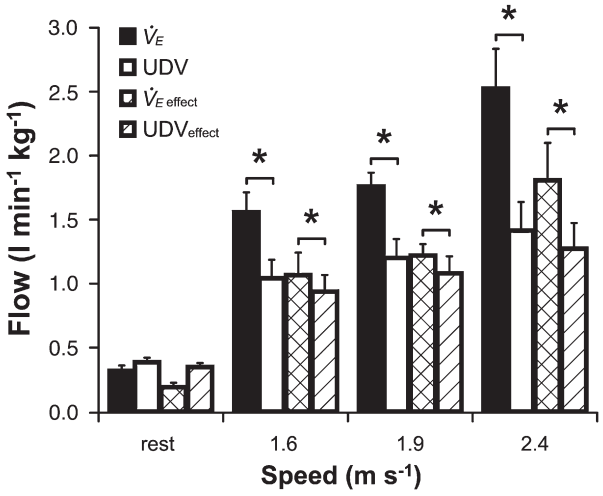

Fig. 3. Self-ventilation and artificial ventilation rates and effective ventilation rates at rest and during running. Mean ventilation rates while breathing ($\dot{V}_E$, dark bars), artificial ventilation rates required for the birds to stop breathing (UDV, light bars), estimated effective ventilation while breathing ($\dot{V}_{E\ effect}$, dark grey bars), and estimated effective UDV rates (UDV_{effect}, light grey bars) at rest and each of three running speeds for all individuals ($N=4$). UDV tended to be higher than $\dot{V}_E$ and UDV_{effect} tended to be higher than $\dot{V}_{E\ effect}$ at rest; across running speeds $\dot{V}_E$ was significantly higher than UDV (Wilcoxon Sign-Rank: $p=0.0005$) and $\dot{V}_{E\ effect}$ was significantly higher than UDV_{effect} ($p=0.0005$). Error bars show S.E.

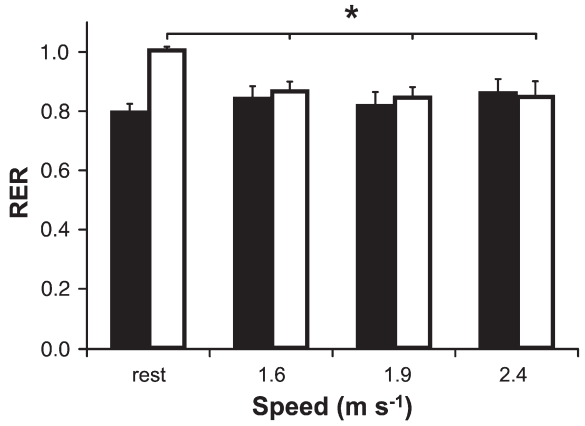

Fig. 5. Respiratory exchange ratios while breathing and apneic at rest and during running. Mean respiratory exchange ratios ($RER=\dot{V}_{CO_2}\cdot\dot{V}_{O_2}^{-1}$) while breathing normally (dark bars) and while apneic due to artificial ventilation (UDV, light bars) for all individuals ($N=4$) at rest and during running. Resting UDV RER tended to be higher than all other values. Breathing RER did not differ across treatment (Friedman's test: $p=0.27$). UDV RER changed significantly with treatment ($p=0.048$). Breathing RER was significantly different than paired UDV RER across all speeds (Wilcoxon Sign-Rank: $p=0.0278$). Error bars show S.E.

breathing and UDV trials ($\Delta\dot{V}_{O_2}$) was negatively correlated with relative UDV rate (standard least squares regression: $R^2=0.47$, $p<0.0001$; Fig. 4). *High UDV* rounds (closed symbols, Fig. 4) had the highest relative UDV rates and the greatest increase in $\dot{V}_{O_2}$ during UDV (most negative $\Delta\dot{V}_{O_2}$). Rounds with relative UDV rates of approximately one (0.9 to 1.1) had a mean $\Delta\dot{V}_{O_2}$ indistinguishable from zero (-0.5 ± 0.9 mL O_2 min^{-1} kg^{-1}).

3.5. RER differed between resting and running

Interestingly, the mean relationship between CO_2 loss and O_2 consumption (RER) during resting UDV (1.01 ± 0.01) was higher than the RER while breathing normally at rest (0.80 ± 0.03), breathing normally and running (0.84 ± 0.02) or while running during UDV (0.85 ± 0.02, Fig. 5). However, there was not sufficient power to show that resting UDV RER was statistically different from the RER of all other treatments using non-parametric statistics. Breathing RER was not significantly different across treatment (Friedman's test: $p=0.27$), while UDV RER differed significantly across treatment ($p=0.048$). Additionally, breathing RER was significantly different than paired UDV RER across all speeds (Wilcoxon Sign-Rank: $p=0.0278$). Both significant effects are likely due to the high resting UDV RER.

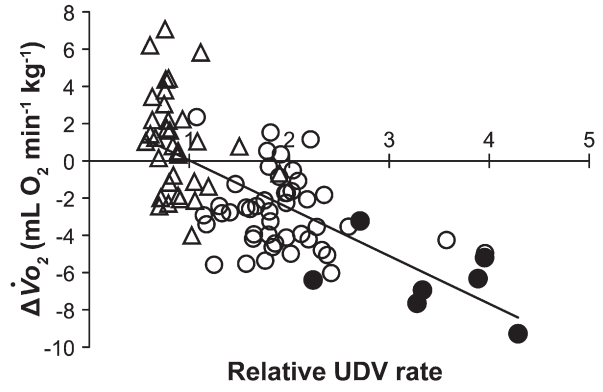

Fig. 4. The change in oxygen consumption varies with relative artificial ventilation rate. The relationship between relative UDV rate (effective UDV rate divided by effective ventilation rate of the paired breathing trial) and the change in oxygen consumed between breathing and paired artificially ventilated trial ($\Delta\dot{V}_{O_2}$). Triangles represent running data rounds, circles represent resting rounds. Open symbols are data from rounds with minimum UDV rates, closed circles are from resting rounds with elevated UDV rates. Relative UDV rates <1 indicate under-ventilation during UDV, while relative UDV rates >1 indicate over-ventilation. $\Delta\dot{V}_{O_2}$ decreased with increasing relative UDV rate (standard least squares regression: $R^2=0.47$, $p<0.0001$).

4. Discussion

4.1. Cost of ventilation in birds

The results of this study are consistent with a low cost of ventilation in birds. During running, oxygen consumption decreased significantly when subjects were not breathing, indicating a cost of ventilation during running of $1.43\pm0.62\%$ of total running metabolism or 0.48 ± 0.21 mL O_2 (L ventilated)$^{-1}$ (Table 2). The CO_2 lost during UDV decreased with running speed. Thus, if CO_2 loss is related to the increase in oxygen consumption with UDV at rest (see Section 4.3), any increase in oxygen consumption with UDV during running should be minimized at the highest running speed, and the cost of ventilation measured at this speed ($3.6\pm0.9\%$ of total running $\dot{V}_{O_2}$; 1.14 ± 0.37 mL O_2 (L vent.)$^{-1}$) may be closest to the true cost of ventilation. Thus the mean across all speeds may underestimate of the cost of ventilation in running guinea fowl. If the cost of ventilation was high in birds, as we hypothesized, the metabolic savings during UDV would likely have been large compared to the cost of replenishing lost CO_2, and resting birds would have exhibited a reduction in metabolism during UDV.

4.2. Comparison with cost of ventilation in other vertebrates

The cost of resting ventilation is generally thought to be a few percent of resting $\dot{V}_{O_2}$ in amniotes. However, high costs of ventilation have been reported in some non-avian sauropsids (Table 3), including the only other study we are aware of in which ventilation was changed with UDV (Kinney and White, 1977). The reason for the high cost in turtles measured by UDV is unclear, but other authors (Jackson et al., 1991) have suggested methodological problems with this study (not specifically related to UDV itself).

The cost of ventilation has often been shown to increase with increasing ventilation rate (Table 3) and this has been especially apparent in studies measuring the cost of ventilation during running; costs of ventilation have been reported from 1.5 to 12 mL O_2 (L vent.)$^{-1}$ and from 3 to 18% of total running $\dot{V}_{O_2}$ (Table 3; Marconi et al., 1982; Roussos and Zakynthinos, 1995; Dempsey et al., 1996; Marks et al.,

2005; Vella et al., 2006). The mean cost of ventilation reported here is within the range of those reported for both resting and running mammals and is similar to the cost estimated from the increase in blood flow with exercise in guinea fowl after locomotor and heart muscle blood flows were removed (Ellerby et al., 2005).

The mass of the ventilatory system is large in birds, so how do birds achieve a low cost of ventilation? Because gravity tends to pull the sternum down, inspiration may be largely produced through eccentric contractions of ventilatory muscles acting to break the fall of the sternum. Eccentric muscle contractions produce mechanical work more economically than concentric contractions. It is also possible that birds do not actively affect inspiration but allow gravity to lower the sternum and break the fall of the sternum by storing elastic strain energy in the ventilatory muscles that can then be recovered to lift the sternum during expiration.

4.3. Critique of UDV method

The results of this study suggest that the UDV method may not be appropriate to measure the cost of ventilation in resting birds. Instead of metabolism decreasing during UDV induced apnea, it increased. The increase in $\dot{V}_{O_2}$ when resting birds were apneic was not found to be attributable to pain or stress during UDV, depressed metabolism during breathing because of the preceding hypercapnic air trial, or the cooling effect of UDV. However, increasing the rate of UDV 1.5- and 2-fold over the rate necessary to stop ventilation did increase UDV metabolism (Fig. 2). In this context, it may be significant that the UDV rate required to stop ventilation at rest was higher than the minute ventilation of the paired breathing trial (Fig. 3). Thus, during UDV, birds appear to have been over-ventilated compared to their self-ventilation rates at rest. Additionally, as the relative UDV rate (UDV rate divided by normal $\dot{V}_E$) increased, the change in oxygen consumption between breathing and UDV trials became greater (i.e. UDV metabolism increased; Fig. 4, circles). In contrast, running birds were under-ventilated compared to the minute ventilation in paired breathing trials (Fig. 3), and there was no clear relationship between relative UDV rate and change in oxygen consumption for running rounds (triangles, Fig. 4). This may indicate that hypocapnic alkalosis was less important during running, possibly due to lactic acid alleviating the effect of hypocapnia.

Although multiple possibilities may explain the increase in $\dot{V}_{O_2}$ during UDV in resting birds, we suspect that it was the result of a high loss of CO_2, and that this loss of CO_2 induced some unidentified energy consuming cycle to replenish lost CO_2 for pH homeostasis. In mammals, hypocapnia causes an increase in whole body oxygen consumption and an increase in lactic acid production through glycolysis, which helps correct body pH (Siesjö et al., 1974; Harken, 1976; Theye et al., 1977; Bharma and Milsom, 1993). Injection of sodium bicarbonate in anesthetized dogs (Gesell et al., 1930) produced results similar to UDV in our study. Both manipulations produced an increase in $\dot{V}_{O_2}$ and a greater increase in $\dot{V}_{CO_2}$ (although in our study the magnitude of increase in $\dot{V}_{CO_2}$ decreased with increasing exercise load). In the study by Gesell et al. (1930), the increase in metabolism was coupled with an increase in pH, and an increase in blood levels of lactic acid. These results are consistent with our hypothesis that UDV produces alkalosis that must be rectified via an increase in metabolism.

4.4. Limitations to measuring cost of ventilation

Many of the approaches used to estimate the cost of ventilation have methodological problems, including those of this study. Using exercise

Table 3
Previously reported cost of ventilation for a variety of species at rest (top) and during running (bottom).

Species	$\Delta\dot{V}_{O_2} \cdot \Delta\dot{V}_E^{-1}$ (mL O_2 L^{-1})	$\%\Delta\dot{V}_{O_2}$	Ventilation changed via	Reference
Florida cooter				
Pseudemys floridana	4.7	10%	UDV	Kinney and White (1977)
Painted turtle				
Chrysemys picta bellii		<1%	↑CO_2	Jackson et al. (1991)
Tegu				
Tupinambis merianae	−1.9	−5%	↑CO_2	Skovgaard and Wang (2004)
	6	17%	↓O_2	Skovgaard and Wang (2004)
American alligator	−0.7	−1.5%	↑CO_2	Wang and Warburton (1995)
Alligator mississippiensis	5.3	12.9%	↓O_2	Wang and Warburton (1995)
		0	↑CO_2, ↓O_2, vagotomy	Skovgaard and Wang (2007)
Human				
Homo sapiens	3.1–3.3		Various methods	Murray (1959)
	0.4–8.3[a]		Hypervent., ↑ dead space	[Roussos and Zakynthinos (1995)]
	2.8		Hypervent.	Anholm et al. (1987)
	0.3–0.8	1–2%	↑CO_2	[Roussos and Zakynthinos (1995)]
	0–25[a]		↑CO_2	Kaminski et al. (1982)
Ox				
Bos taurus	3–4		↑CO_2	Hales and Findlay (1968)
Pony				
Equus caballus	2.5–7[a]		↑CO_2	Kaminski et al. (1982)
	4.1		↑CO_2	Kaminski et al. (1985)
Human	1.5–2.0[b]	3–5%[b]	Hypervent. as in exercise	[Dempsey et al. (1996)]
Homo sapiens	>3.0[b]	8–10%[b]		
	1.5–4.4[b]		Exercise	[Roussos and Zakynthinos (1995)]
	4–5[b]	up to 18%[b]	Hypervent. as in exercise	Marks et al. (2005)
	2.1–2.7	5–18%[b]	Hypervent. as in exercise	Vella et al. (2006)
Dog				
Canis familiaris	12		Exercise	Marconi et al. (1982)
Horse				
Equus caballus		4.5–12%[b]	Exercise	Art et al. (1990)
Guinea fowl				
Numida meleagris		2%[c]	Exercise	Ellerby et al. (2005)

References in brackets are reviews. *Abbreviations*: $\Delta\dot{V}_{O_2} \cdot \Delta\dot{V}_E^{-1}$, unit cost of ventilation, change in oxygen consumption per change in ventilation; $\%\Delta\dot{V}_{O_2}$, percent change in $\dot{V}_{O_2}$ with change in ventilation; UDV, unidirectional artificial ventilation; ↑CO_2, breathing hypercapnic air; ↓O_2, breathing hypoxic air; hypervent., voluntary hyperventilation; ↑ dead space, increased dead space.

[a] Cost decreased with increasing proportion of CO_2 in inspired air.

[b] Cost increased with ventilation rate.

[c] Percent change in blood flow not $\dot{V}_{O_2}$.

to increase ventilation adds the difficulty of the synergistic effects of the coordination of ventilation and exercise. Increases in metabolism concurrent with exercise hyperpnoea may over- or underestimate the cost of ventilation depending on how it is measured. Other studies have reported results consistent with changes in whole body metabolism with hypercapnia and hypoxia. For example, the non-avian sauropsid studies, which used hypercapnia to increase ventilation, reported lower costs of ventilation than studies that used hypoxia to increase ventilation, and the cost of increasing ventilation through breathing hypercapnic air decreased with increasing proportion of CO_2 in the inspired air in humans and ponies (Table 3). These results indicate that increased CO_2 inhalation reduces non-ventilatory metabolism. In this study, the cost of ventilation tended to increase with running speed and ventilation rate, but the increase was not significant (Table 2). Cost of ventilation is often reported to increase with increasing ventilation rate in mammals (Table 3). This increase in cost may, indeed, be due to an elevated cost of high breathing rates, or it may be a by-product of increased metabolism induced by hypocapnic alkalosis with increasing ventilation rate. Further studies are needed to ascertain the change in non-ventilatory metabolism due to hypo- or hypercapnia (Skovgaard and Wang, 2007).

Any study that measures the cost of ventilation using methods that influence CO_2 loss or pH is likely to introduce metabolic artifacts. Care must be taken when using any manipulation that changes whole body CO_2 loss or pH, while measuring changes in metabolism. Studies that manipulated ventilation rate in humans through voluntary hyperventilation while providing subjects with hypercapnic air may mitigate the effect of the increase in metabolism caused by hypocapnia and may yield reasonable approximations for cost of ventilation (Table 3), as would studies that checked their results by changed ventilation using multiple methods (Murray, 1959; Wang and Warburton, 1995; Skovgaard and Wang, 2004; Skovgaard and Wang, 2007). UDV may be as accurate as many previously used methods, since all methods that change ventilation, will necessarily and unavoidably change whole body pH and metabolism.

Given the limitations of modern methods, it may not be possible to accurately measure the cost of ventilation. Manipulation of ventilation rate impacts retention or loss of CO_2, which influences pH. Because of if its importance to all cell processes, the body is expected to tightly regulate pH. Thus, manipulation of ventilation simultaneously changes the work of ventilation and the energy consumed by pH homeostatic mechanisms, both of which impact metabolism. Unless methods can be found to control pH, the cost of ventilation may be irresolvable.

5. Conclusions

The results of this study suggest that the cost of ventilation in birds is low. During running, the unidirectional artificial ventilation method provided cost of ventilation estimates of $1.44 \pm 0.81\%$ of total running $\dot{V}_{O_2}$ and 0.46 ± 0.30 mL O_2 (L ventilated)$^{-1}$. These values are within the range of costs of ventilation reported previously for mammals, including studies that attempted to control for changes in whole body pH. Nevertheless, the UAV method could not be used to measure the cost of ventilation in resting guinea fowl because metabolism increased during UDV. This study raises interesting questions about how non-ventilatory metabolism changes with hypocapnia. It appears that metabolism increased during UDV due to hypocapnic alkalosis, although this hypothesis remains to be tested. Measuring cost of ventilation is confounded by the manipulation of ventilation rate, which unintentionally changes whole body CO_2 loss and pH, and, hence, inextricably changes metabolism.

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