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Ventilatory and metabolic responses of burrowing owls, *Athene cunicularia*, to moderate and extreme hypoxia: Analysis of the hypoxic ventilatory threshold *vs.* hemoglobin oxygen affinity relationship in birds ☆

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Abstract

We measured ventilation, oxygen consumption and blood gases in burrowing owls (*Athene cunicularia*) breathing moderate and extreme hypoxic gas mixtures to determine their hypoxic ventilatory threshold (HVT) and to assess if they, like other birds and mammals, exhibit a relationship between HVT and hemoglobin O_2 affinity (P_{50}) of their blood. An earlier report of an attenuated ventilatory responsiveness of this species to hypoxia was enigmatic given the low O_2 affinity (high P_{50}) of burrowing owl hemoglobin. In the current study, burrowing owls breathing 11% and 9% O_2 showed a significantly elevated total ventilation. The arterial partial pressure of oxygen (PaO_2) at which ventilation is elevated above normoxic values in burrowing owls was 58 mm Hg. This threshold value conforms well to expectations based on the high P_{50} of their hemoglobin and the HVT *vs.* P_{50} relationship for birds developed in this study. Correcting for phylogenetic relatedness in the multi-species analysis had no effect on the HVT *vs.* P_{50} relationship. Also, because burrowing owls in this study did not show a hypometabolic response at any level of hypoxia (even at 9% O_2); HVT described in terms of percent change in oxygen convection requirement is identical to that based on ventilation alone.

Keywords: *Athene cunicularia*; Burrowing owl; Hypoxia; Hypoxic ventilatory threshold; Metabolism; Oxygen convection requirement; Phylogenetically independent contrasts; Ventilation

1. Introduction

Comparative studies with birds and mammals have demonstrated an underlying relationship between hypoxic ventilatory threshold (typically defined as the arterial partial pressure of oxygen, PaO_2, at which ventilation is elevated above normoxic values by 10%) and hemoglobin O_2 affinity (Black and Tenney, 1980; van Nice et al., 1980; Boggs and Birchard, 1983; Boggs, 1995). Species with low hemoglobin O_2 affinity (i.e., high P_{50}) have higher hypoxic ventilatory thresholds (HVT) and vice versa. Basically, total ventilation ($\dot{V}_E$) is significantly elevated when PaO_2 declines sufficiently to reduce the oxygen saturation (SaO_2) of an animal's hemoglobin to between 0.6 and 0.85 (see Table 1 in Boggs, 1995).

In light of the HVT *vs.* P_{50} relationship, we should have observed a significant increase in the ventilation of burrowing owls (*Athene cunicularia*) breathing 13% O_2 in our earlier study on the hypoxic ventilatory response of this species (Boggs and

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Kilgore, 1983) given the relatively low O_2 affinity (P_{50}=42–45 mm Hg; 41 °C; pH=7.5) of their hemoglobin (Boggs et al., 1983; Maginniss and Kilgore, 1989). Two lines of evidence support this contention. First, other birds with similarly low hemoglobin O_2 affinities, the ring-necked pheasant (P_{50}=42.4 mm Hg; 41 °C; pH=7.5) and the Pekin duck (P_{50}=42.9 mm Hg; 41 °C; pH=7.5) exhibit a significantly elevated ventilation at inspired levels of oxygen considerably greater (PIO_2=116 mm Hg and 105 mm Hg, respectively) than that of owls breathing 13% O_2 in our earlier study (PIO_2=82 mm Hg) (Black and Tenney, 1980; Boggs and Birchard, 1983). Secondly, in a study in which blood gases of burrowing owls, but not ventilation, were measured, the mean PaO_2 of burrowing owls breathing a hypoxic gas mixture containing 13% O_2 (PIO_2=88 mm Hg) was 56 mm Hg (Kilgore et al., 1994). At this arterial partial pressure of oxygen, hemoglobin of burrowing owls should be only 69% saturated (calculated from Eq. 1 in Maginniss and Kilgore, 1989), a saturation sufficiently low to stimulate ventilation according to the HVT *vs.* P_{50} relationship (Boggs, 1995).

Why we did not observe elevated total ventilation in burrowing owls breathing 13% O_2 in our earlier study (Boggs and Kilgore, 1983) is not clear. One possible explanation is that the relationship between hypoxic ventilatory threshold and hemoglobin O_2 affinity simply may not hold for burrowing owls (Boggs et al., 1983; Kilgore et al., 1992). Burrowing owls naturally encounter hypoxic environments (Birchard et al., 1984) and thus may have a blunted chemosensitivity to hypoxia. Alternatively, the small number of owls studied (n=5), natural variability in total ventilation measurements and the lack of PaO_2 data in the earlier ventilation study (Boggs and Kilgore, 1983) may well have prevented our detection of the threshold value. Indeed, in the same study in which blood gases were measured (Kilgore et al., 1994), it was observed that the arterial partial pressure of CO_2 ($PaCO_2$) of owls breathing 13% O_2 mixtures was significantly reduced compared to normoxic values, indicating a marked hyperventilation. A primary goal of the current study, therefore, was to determine the actual hypoxic ventilatory threshold of burrowing owls and then to analyze the HVT *vs.* P_{50} relationship in birds with regards to the new information from the present study, unpublished data from our laboratory on Japanese quail (*Coturnix japonica*), and available data from the literature. Given the robust relationship between HVT *vs.* P_{50} in other birds and mammals, we hypothesized that burrowing owls would likewise conform to this relationship. In light of concerns regarding the lack of independence in multi-species analyses, we also analyzed the HVT *vs.* P_{50} relationship using the phylogenetically independent contrast method.

Furthermore, because oxygen consumption was unchanged in burrowing owls breathing hypoxic mixtures >13% O_2 (Boggs and Kilgore, 1983), it is not known if they, like most mammals (Frappell et al., 1992; Mortola, 1993; Gautier, 1996; Walsh et al., 1996) and some birds, show a reduction in metabolism when exposed to more extreme levels of inspired hypoxia. A hypometabolic response to hypoxia would modify the hypoxic ventilatory response of burrowing owls through changes in ventilation relative to metabolic demand (Boggs, 1995). A second goal of the present study, then, was to determine the oxygen convection requirement (the ratio of total ventilation to oxygen consumption; $\dot{V}_E/\dot{V}O_2$) of burrowing owls exposed to modest and severe levels of hypoxia.

To meet both these goals, we measured ventilation, oxygen consumption and blood gases in nine burrowing owls breathing moderate to extreme hypoxic gas mixtures ($FIO_2 \leq 0.13$).

2. Materials and methods

2.1. Animals and animal preparation

The mean body mass (±S.D.) of the adult burrowing owls (*Athene cunicularia*; 4 males, 5 females) used in this study was 146±5 g. All owls were collected from natural populations in Kansas, USA. Permits authorizing the collection and possession of owls were obtained from the US Fish and Wildlife Service and the Kansas Wildlife and Parks Commission. In the laboratory, birds were housed individually and fed freshly killed mice once daily. While in captivity (for up to 9 months) all owls maintained their body mass and appeared to be healthy. At the end of our experiments surviving owls (6 of 9) were laparotomized to determine their gender and after recovery were donated to a captive breeding program.

Prior to each experiment, owls were restrained in dorsal recumbency and a local anesthetic (2% lidocaine HCl) was infiltrated at the site of cannulation. A polyethylene catheter (PE 50) was then inserted into either the right or left brachial artery. The animal was allowed to recover for at least 2 h before measurements were taken.

2.2. Measurements

Tidal volume (V_T) and breathing frequency (f_R) of unrestrained burrowing owls breathing various hypoxic gas mixtures were measured in a barometric plethysmograph (Drorbaugh and Fenn, 1955). Pressure deflections within the plethysmograph were obtained with a Validyne DP45-16 pressure transducer and recorded on an oscillographic recorder (Gould, 2200). Volume of the empty plethysmographic chamber was 3.7 L. The plethysmograph was positioned within a constant temperature chamber maintained at 22±1 °C (mean± S.D.) during experiments.

Body temperatures (T_b) of owls within the plethysmograph were measured continuously with a shielded 36 Ga. copper–constantan thermocouple inserted at least 2 cm into the bird's lower digestive tract. Air temperature within the plethysmograph was monitored with a YSI thermistor probe. Thermocouples and the thermistor probe were both calibrated with a thermometer traceable to a NIST calibrated thermometer.

Systemic arterial blood pressure of burrowing owls was recorded using a Statham P23ID pressure transducer coupled to an oscillographic recorder. Systolic (SP) and diastolic pressures (DP) were electronically averaged to obtain mean arterial blood pressure (MABP); mean pressures obtained in this fashion closely approximate integrated pressures (Kilgore et al., 1994).

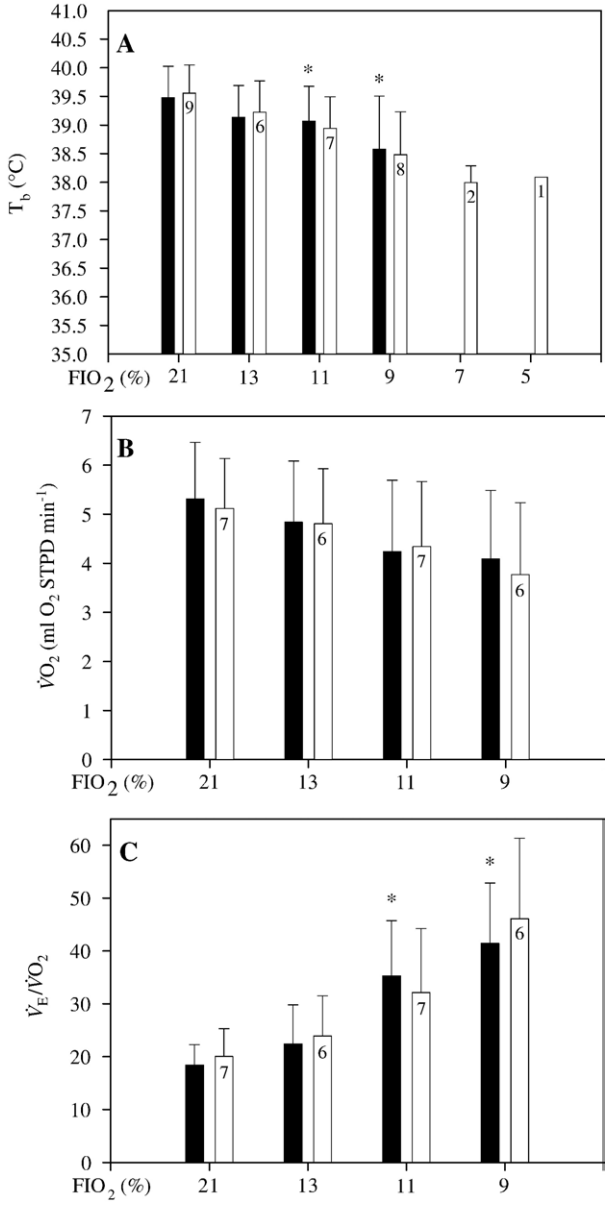

Fig. 1. Mean (±S.D.) body temperature (T_b), rate of oxygen consumption ($\dot{V}O_2$) and oxygen convection requirement ($\dot{V}_E/\dot{V}O_2$) of burrowing owls exposed to room air and declining fractional concentrations of oxygen. Data represented by the solid bars are those statistically analyzed using a one-way repeated measures ANOVA (see Materials and methods). Open bars represent means of all observations, numbers within the vertical bars are the number of animals on which the mean is based. The asterisk identifies significantly ($P<0.05$) higher or lower means compared to the normoxic mean.

Blood samples drawn from the exteriorized arterial catheter were analyzed at 40 °C for pH, PO_2, and PCO_2 with a Radiometer BMS3 Mk2 blood microsystem. Calibration of electrodes and correction of blood gas and pH values to the bird's body temperature followed procedures described previously (Kilgore et al., 1994). After measurements had been obtained, blood was withdrawn from the analyzer, warmed and injected into the bird. Nearly 80% of the volume of blood withdrawn from individual birds during experiments was returned.

Hypoxic gas mixtures were mixed with rotameters. The total flow of gas through the plethysmograph was 2.2 L STPD min^{-1}. All rotameters were calibrated with a NIST certified Vol-U-Meter (Brooks, 1058-7A) at experimental pressures. Gas mixtures were heated and humidified before entering the plethysmograph. Fractional concentrations of oxygen in dry samples of inflow and outflow gases were measured with an applied Electrochemistry (S-3A) oxygen analyzer. Oxygen consumption ($\dot{V}O_2$) was calculated using Withers' (1977) equation 1d assuming a respiratory quotient of 0.85 and changes in the flow rate resulting from ambient water vapor and the birds' evaporative water loss were zero.

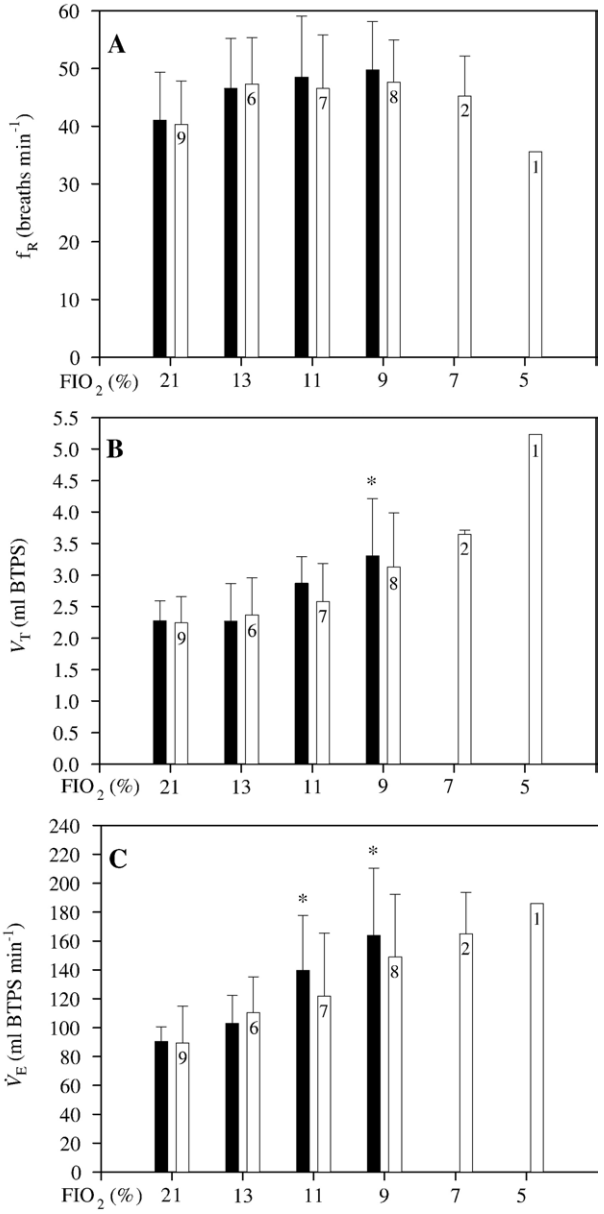

Fig. 2. Mean (±S.D.) respiratory frequency (f_R), tidal volume (V_T) and total ventilation ($\dot{V}_E$) of burrowing owls exposed to room air and declining fractional concentrations of oxygen. Symbols as in Fig. 1.

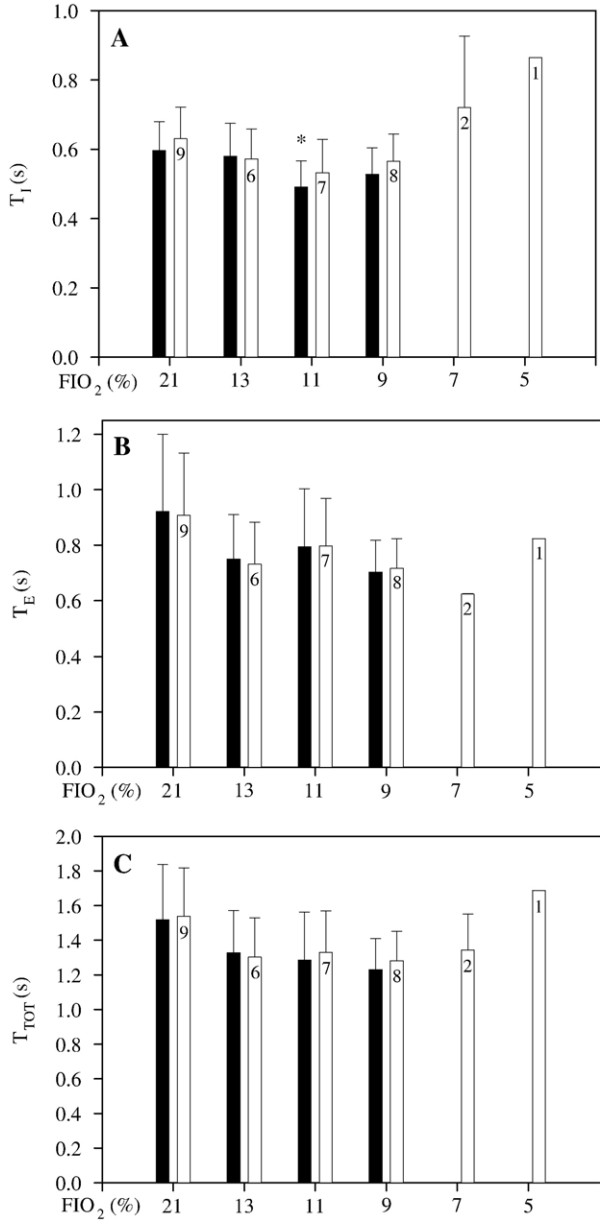

Fig. 3. Mean (±S.D.) inspiratory period (T_I), expiratory period (T_E), and total breath time (T_{TOT}) of burrowing owls exposed to room air and declining fractional concentrations of oxygen. Symbols as in Fig. 1.

The mean (±S.D.) barometric pressure recorded during the owl experiments was 678±4 mm Hg; barometer readings were temperature corrected, but not adjusted for gravity given our proximity to the 45th parallel.

2.3. Experimental protocol

Owls were exposed to two or more hypoxic gas mixtures for at least 15 min each. The order in which each bird was exposed to the treatment gas mixtures was randomized. At the end of each exposure, the plethysmograph was sealed and T_b, chamber temperature, blood pressures, and pressure oscillations within the plethysmograph were recorded. Just prior to obtaining ventilatory measurements, the fractional concentration of oxygen in the incurrent and excurrent air was determined. Following a consistent record of pressure oscillations with minimal drift, several calibration volumes of 0.2 cc were injected into the plethysmograph to coincide with zero flow at the end of inspiration (Bartlett and Tenney, 1970). Near the end of exposure to a particular hypoxic gas mixture, a 120–150 μL arterial blood sample was drawn anaerobically from the catheter and immediately analyzed for PO_2, PCO_2, and pH. Between hypoxic exposures, birds were allowed to breathe room air for 15 min or until blood gases and pH had returned to room air values before being exposed to another hypoxic gas mixture.

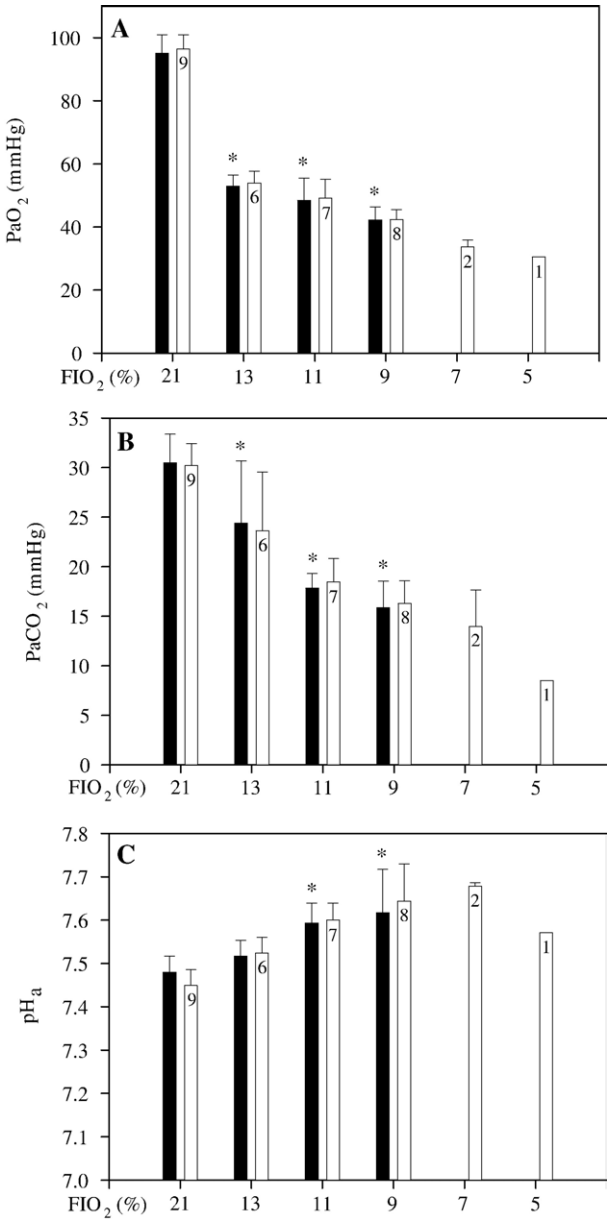

Fig. 4. Mean (±S.D.) partial pressure of oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$) and arterial pH (pH_a) of burrowing owls exposed to room air and declining fractional concentrations of oxygen. Symbols as in Fig. 1.

The packed cell volume of (PCV) of each bird's blood was measured at the beginning and end of experiments using conventional techniques.

2.4. Data analysis

A mean (±S.D.) of 19±3 pressure waves were analyzed from each spirographic recording (Bartlett and Tenney, 1970). The mean tidal volume so obtained was then multiplied by breathing frequency to obtain $\dot{V}_E$.

Data for burrowing owls breathing room air (21% O_2) obtained in this study were compared to those reported in previous studies or allometrically derived values using two-tailed *t*-tests (Sokal and Rohlf, 1995). To determine the effect of hypoxia on the variables measured, data obtained from a sub-set of five of the nine owls studied were analyzed. The five owls included in the statistical analyses were those for which complete data were available at four levels of hypoxia (21%, 13%, 11%, and 9% O_2). These data were analyzed using a one-way repeated measures ANOVA (SigmaStat, Jandel Scientific) and are represented as *solid bars* in Figs. 1 2 3 4, and 5. Values from all owls studied ($n=9$) are presented in Table 1 and Figs. 1 2 3 4, and 5 (*open bars*). It was not possible to statistically analyze the total data obtain from all nine owls in this study because of missing data from four of the birds at one or more treatments. Planned comparisons between means obtained at hypoxic treatments with those at room air were made using Dunnett's method. In two instances where data were not normally distributed or variances were not equal, Friedman's test was used. In all analyses, statistical significance was assumed at a 95% probability level ($\alpha=0.05$).

Ventilatory thresholds were modeled using a piecewise regression technique in which indicator or dummy values are used to fit linear regressions consisting of two pieces (Neter and Wasserman, 1974; Toms and Lesperance, 2003). The PaO_2 at the breakpoint yielding the highest F_{Model} from the ANOVA associated with the regression analysis was assumed to be the threshold. Piecewise regression analyses were performed using SAS JMP-IN (v. 4).

Data relating the hypoxic ventilatory threshold of birds with the O_2 affinity of their hemoglobin were analyzed using a traditional regression analysis. Since some species included in the regression analysis are more closely related than others (Frappell et al., 2001), we also analyzed these data by the method of phylogenetically independent contrasts (Felsenstein, 1985; Frappell et al., 2001; Hempleman et al., 2005).

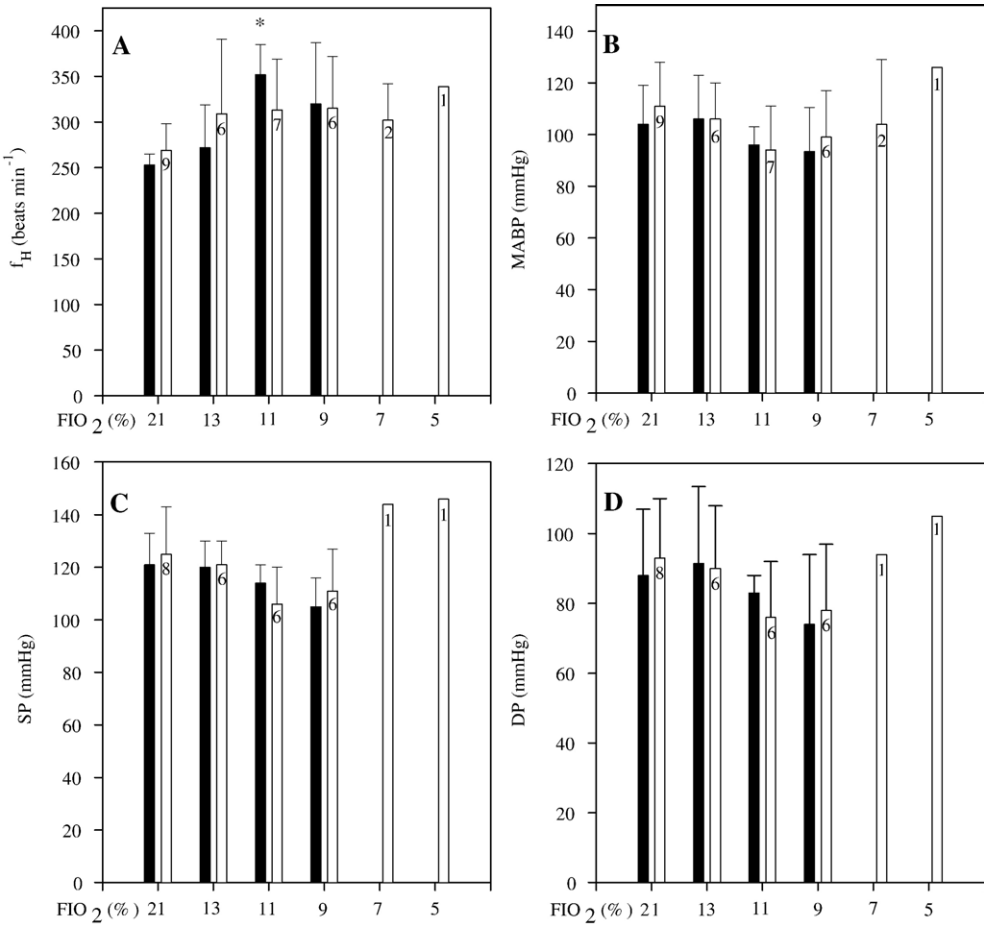

Fig. 5. Mean (±S.D.) heart rate (f_H), arterial blood pressure (MABP), systolic pressure (SP) and diastolic pressure (DP) of burrowing owls exposed to room air and declining fractional concentrations of oxygen. Symbols as in Fig. 1.

Table 1
Body temperature, ventilatory parameters, blood gases, metabolic rate, oxygen convection requirement and cardiovascular parameters of burrowing owls breathing normoxic and moderate to extreme hypoxic gas mixtures

Parameter	21% O_2	13% O_2	11% O_2	9% O_2	7% O_2	5% O_2
T_b (°C)	39.6±0.5 (9,25)	39.2±0.5 (6,7)	38.9±0.6 (7,7)	38.5±0.7 (8,8)	38.0±0.3 (2,2)	38.1 (1,1)
V_T (mL BTPS)	2.2±0.4 (9,25)	2.4±0.6 (6,7)	2.6±0.6 (7,7)	3.1±0.9 (8,8)	3.6±0.7 (2,2)	5.2 (1,1)
f_R (breaths min^{-1})	40±8 (9,25)	47±8 (6,7)	47±9 (7,7)	48±7 (8,8)	45±7 (2,2)	36 (1,1)
$\dot{V}_E$ (mL BTPS min^{-1})	89.5±25.4 (9,25)	110.3±24.9 (6,7)	121.9±43 (7,7)	149.0±43.4 (8,8)	165.0±28.6 (2,2)	185.9 (1,1)
T_I (s)	0.63±0.09 (9,25)	0.57±0.09 (6,7)	0.53±0.10 (7,7)	0.57±0.08 (8,8)	0.72±0.21 (2,2)	0.86 (1,1)
T_E (s)	0.91±0.22 (9,25)	0.73±0.15 (6,7)	0.80±0.17 (7,7)	0.72±0.11 (8,8)	0.62±0.01 (2,2)	0.82 (1,1)
T_{TOT} (s)	1.54±0.28 (9,25)	1.30±0.23 (6,7)	1.33±0.24 (7,7)	1.28±0.17 (8,8)	1.34±0.21 (2,2)	1.69 (1,1)
PaO_2 (mm Hg)	96.5±4.5 (9,18)	53.9±3.8 (6,7)	49.1±6.0 (7,7)	42.4±3.1 (8,8)	33.7±2.3 (2,2)	30.5 (1,1)
$PaCO_2$ (mm Hg)	30.2±2.2 (9,18)	23.6±5.9 (6,7)	18.4±2.4 (7,7)	16.3±2.3 (8,8)	13.9±3.7 (2,2)	8.5 (1,1)
pH_a	7.49±0.04 (9,18)	7.52±0.04 (6,7)	7.60±0.04 (7,7)	7.64±0.09 (8,8)	7.68±0.01 (2,2)	7.57 (1,1)
$\dot{V}O_2$ (mL O_2 STPD min^{-1})	5.12±1.02 (7,19)	4.81±1.12 (6,7)	4.34±1.32 (7,7)	3.77±1.46 (6,6)		
$\dot{V}O_2$/mass (mL O_2 STPD g^{-1} min^{-1})	0.034±0.007 (7,19)	0.033±0.006 (6,7)	0.030±0.010 (7,7)	0.026±0.013 (6,6)		
$\dot{V}_E/\dot{V}O_2$	20.0±5.3 (7,19)	23.9±7.6 (6,7)	32.1±12.1 (7,7)	46.1±15.3 (6,6)		
Systolic pressure (mm Hg)	125±18 (8,22)	121±9 (6,7)	106±14 (6,6)	111±16 (6,6)	144 (1,1)	146 (1,1)
Diastolic pressure (mm Hg)	93±17 (8,22)	90±18 (6,7)	76±16 (6,6)	78±19 (6,6)	94 (1,1)	105 (1,1)
MABP (mm Hg)	111±17 (9,25)	106±14 (6,7)	94±17 (7,7)	99±18 (6,6)	104±25 (2,2)	126 (1,1)
f_H (beats min^{-1})	269±29 (9,25)	309±82 (6,7)	313±56 (7,7)	315±57 (6,6)	302±40 (2,2)	339 (1,1)

All values are means±S.D. Numbers in parentheses are numbers of burrowing owls and numbers of observations, respectively.

Comparison of slopes from these two separate analyses was made using a two-tailed *t*-test (Sokal and Rohlf, 1995).

Values in the text are means±S.D.

3. Results

3.1. Normoxic values

The mean resting body temperature, ventilatory parameters, blood gases, metabolic rate, oxygen convection requirement and cardiovascular parameters of burrowing owls breathing room air obtained in this study are presented in Table 1.

3.2. Cardiorespiratory responses of burrowing owls to moderate and extreme hypoxia

The body temperature, respiratory, metabolic and cardiovascular responses of all burrowing owls exposed to hypoxic gas mixtures are summarized in Table 1 and Figs. 1, 2, 3, 4, and 5.

Hypoxia induced a 1.5 °C decline in body temperature over the range of hypoxia owls were exposed to in this study (Table 1; Fig. 1A, *open bars*). The mean body temperature of the subset of owls included in the statistical analyses (see Materials and methods) at 11 and 9% O_2 (39.1±0.6 °C and 38.6±0.9 °C, respectively) are significantly less ($P=0.001$) than that observed in normoxia (39.5±0.6 °C) (Fig. 1A, *solid bars*). There was a further decline in body temperature in the two owls exposed to the most extreme levels of hypoxia (Table 1; Fig. 1A, *open bars*). Metabolism of burrowing owls was not statistically affected by any level of hypoxia (Fig. 1B, *solid bars*).

Respiratory frequency increased somewhat with exposure to hypoxia from a mean of 41±8 breaths min^{-1} to 50±8 breaths min^{-1} (Fig. 2A, *solid bars*). In the single owl breathing 5% O_2, f_R declined to 36 breaths min^{-1}. Due to the high variance in the measurements of respiratory frequency no statistical significance could be detected in these data ($P=0.07$). Tidal volume varied significantly with hypoxia ($P=0.028$; Fig. 2B, *solid bars*). However, only in owls breathing 9% O_2 (3.3±0.9 mL BTPS) was tidal volume significantly ($P<0.05$) elevated relative to normoxia (2.3±0.3 mL BTPS). In owls exposed to even more extreme hypoxia ($FIO_2=0.07$ and 0.05) V_T was further increased (Table 1; Fig. 2B, *open bars*). Increases in both f_R and V_T produced significantly elevated total ventilation ($\dot{V}_E$) at levels of hypoxia below 13% O_2 ($P=0.009$). At a FIO_2 of 0.11, mean $\dot{V}_E$ was 139.7±38.1 mL BTPS min^{-1}, while at 9% O_2 $\dot{V}_E$ averaged 163.9±46.6 mL BTPS min^{-1} (Fig. 2C, *solid bars*). Total ventilation continued to increase at more extreme levels of hypoxia; $\dot{V}_E$ was 185.9 mL BTPS min^{-1} in the owl exposed to 5% O_2 (Table 1; Fig. 2C, *open bars*).

The oxygen convection requirement of owls exposed to extreme hypoxia was significantly elevated ($P=0.002$). The mean oxygen convection requirement of owls breathing 11% and 9% O_2 were significantly different ($P<0.05$) from the normoxic mean (Table 1; Fig. 1C).

Respiratory pattern of burrowing owls was only minimally affected by breathing hypoxic gas mixtures (Table 1, Fig. 3A–C). Although inspiratory period (T_I) was significantly altered by hypoxia ($P=0.038$), only the contrast between the mean T_I at 11% and that at 21% O_2 was significantly different (Fig. 3A, *solid bars*). Expiratory period (T_E) and total breath time (T_{TOT}) (Fig. 3B and C, *solid bars*) were not affected by hypoxia ($P=0.078$ and $P=0.160$, respectively).

Burrowing owls exposed to hypoxia developed a significant hypoxemia ($P<0.001$; Table 1, Fig. 4A, *solid bars*). Arterial partial pressure of oxygen was significantly reduced ($P<0.05$) at all levels of hypoxia included in the statistical analysis (Fig. 4A, *solid bars*). The marked reduction in PaO_2 was accompanied by a concomitant drop in $PaCO_2$ ($P<0.001$; Table 1; Fig. 4B, *solid bars*). Correspondingly, there was a significant effect of hypoxia on arterial pH ($P=0.01$). The mean pH_a of owls exposed to 11% and 9% O_2 was significantly

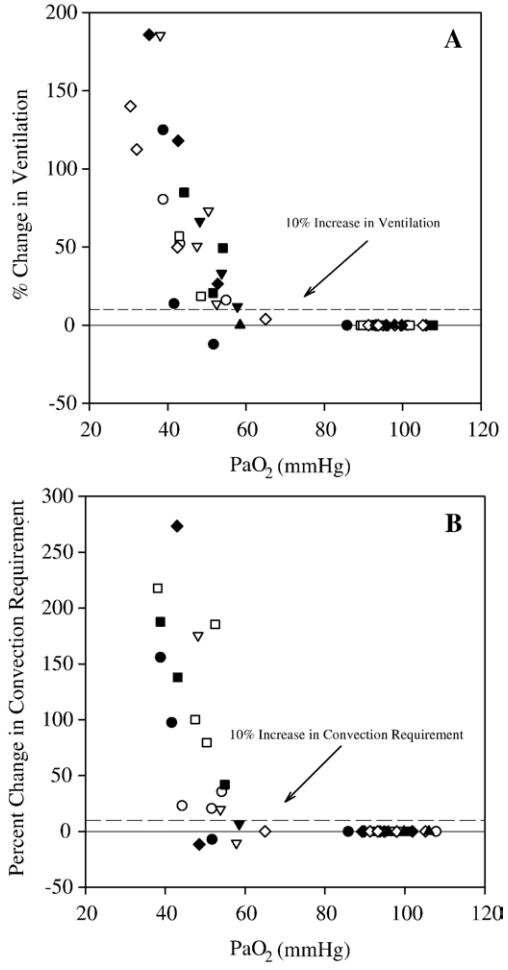

Fig. 6. A. Relationship between the increase in ventilation of burrowing owls breathing air or hypoxic gas mixtures (expressed as a percent change above normoxic values) and the partial pressure of oxygen in their blood (PaO_2). B. Relationship between increase in oxygen convection requirement of burrowing owls breathing air or hypoxic gas mixtures (expressed as a percent change above normoxic values) and the partial pressure of oxygen in their blood (PaO_2). Data for all nine burrowing owls studied are included; data for an individual burrowing owl are identified by a specific symbol.

greater than that when they breathed air ($P<0.05$) (Fig. 4C, *solid bars*). Without further assessment, the decline in pH_a at the most extreme hypoxic treatment (5% O_2, Fig. 4C, *open bar*), relative to the mean pH_a value of owls breathing 7% O_2, is difficult to interpret, but this owl may have been relying on anaerobic metabolism at this extreme level of hypoxia.

Heart beat frequency was significantly affected by hypoxia ($P=0.047$) with the mean heart rate of owls exposed to 11% oxygen significantly greater than that of owls breathing room air (Table 1; Fig. 5A, *solid bars*). Hypoxia had no effect on mean arterial blood pressure of burrowing owls ($P=0.381$; Fig. 5B, *solid bars*) or on systolic or diastolic pressure ($P=0.163$ and $P=0.157$, respectively; Fig. 5C and D).

3.3. Hypoxic ventilatory threshold of burrowing owls

Applying the typical definition of the hypoxic ventilatory threshold, ventilation in burrowing owls is increased by 10% or more at a PaO_2 of approximately 60 mm Hg (Fig. 6A). Note the rather steep increase in ventilation once threshold has been reached (Fig. 6A). Ventilation was close to triple normoxic values ($\Delta\dot{V}_E=200\%$) at a PaO_2 of 40 mm Hg or less than 20 mm Hg below threshold. Based on the piecewise regression model, the PaO_2 representing the threshold value was 58 mm Hg which conforms closely to that based on an incremental increase in ventilation (Fig. 6A). In fact, the total ventilation of the owl with a PaO_2 of 57.8 mm Hg was 11.9% above its normoxic value.

Table 2
Hypoxic ventilatory threshold (PaO_2 at which ventilation is elevated 10% above normoxic values) and P_{50} for species included in the traditional and phylogenetically corrected least-squares regression analyses (Fig. 7)

Species	PaO_2 (mm Hg)	P_{50} (mm Hg)	Literature source(s)*
Rhea *Rhea americana*	48	30.5	Boggs and Birchard (1983)
Japanese quail *Coturnix japonica*	56	42.9	Bavis and Kilgore, unpublished data; Baumann and Baumann (1977)
Ring-necked pheasant *Phasianus colchicus*	70	42.4	Boggs and Birchard (1983)
Bar-headed goose *Anser indicus*	38	27.2	(Black and Tenney, 1980; Black et al., 1978)
Pekin duck *Anus platyrhynchos*	70	42.6	(Black and Tenney, 1980; Black et al., 1978)
Burrowing owl *Athena cunicularia*	58	42.3	Present study; Maginniss and Kilgore (1989)

*PaO_2 values were obtained from the first literature source; P_{50} from the second. If only one source is given, both values were obtained from that source.

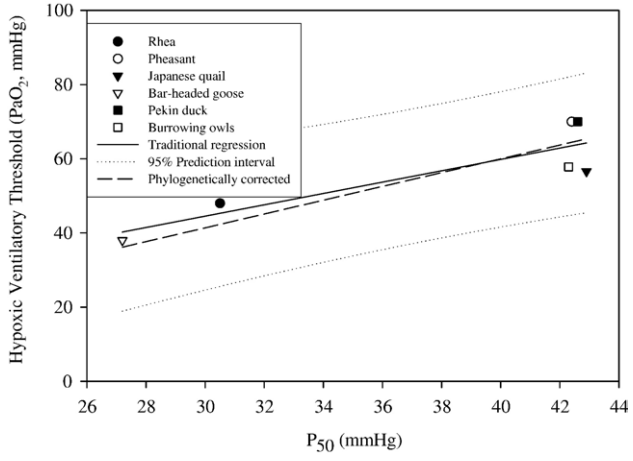

Fig. 7. Relationship between the hypoxic ventilatory threshold and hemoglobin O_2 affinity in birds. Species values are from the literature, the current study and unpublished data (Table 2). The equation for the traditional least-squares line (solid line) is: HVT = 1.53 (±0.44) P_{50} − 1.32 (±16.79). Dotted lines delimit the 95% prediction interval for the traditional regression line. The equation for the least-squares line corrected for phylogenetic relatedness (dashed line) is: HVT = 1.86 (±0.40) P_{50} − 14.37 (±16.10). Values in parentheses are one standard error of the statistic.

4. Discussion

4.1. Comparison of cardiorespiratory data with published values and allometric predictions

Tidal volumes (V_T) of burrowing owls breathing room air in the present study (2.2±0.4 mL BTPS, Table 1) are not significantly different ($0.4>P>0.05$) from those (2.6±0.7 mL BTPS) reported by Souza (1988) at similar air temperatures or predicted values (2.9 and 2.6 mL BTPS) based on traditional and phylogenetically corrected allometric equations in Frappell et al. (2001). However, the mean breathing frequency of owls in the current study (40±8 breaths min^{-1}, Table 1) is significantly different ($P<0.01$) from the mean respiratory frequency of owls (23±3 breaths min^{-1}) in Souza's study. Based on traditional and phylogenetically corrected allometric analysis, owls in the present study would be expected to have respiratory frequencies of 33 and 34 breaths min^{-1}, respectively. The mean breathing frequencies of owls in this study breathing room air are not different from these expected values ($0.5>P>0.2$). Some, but certainly not all of the difference in the empirically determined values (present study and that of Souza, 1988) may result from the difference in the body size of owls in the two studies (146 *vs.*160 g). Souza's frequency values, however, are quite low for the animal's body mass based on allometric predictions (32 breaths min^{-1}; Frappell et al., 2001).

The mean ventilation of burrowing owls reported in Table 1 is not significantly different ($0.9>P>0.05$) from those (97 and 89.5 mL BTPS min^{-1}) predicted by traditional or phylogenetically corrected allometry (Frappell et al., 2001), respectively, and is likewise not different ($P>0.05$) from the mean value reported for burrowing owls by Souza (1988).

For burrowing owls breathing room air, mean T_b (39.6± 0.5 °C) was slightly, but significantly ($P<0.05$) lower than the body temperatures reported previously for burrowing owls (Boggs and Kilgore, 1983; Kilgore et al., 1994), 40.2 and 40.5 °C, respectively. Mean oxygen consumption, however, is not significantly different ($P>0.05$) from published values measured at similar air temperatures (20–25 °C) (Coulombe, 1970; Boggs and Kilgore, 1983) or from values predicted by allometry ($0.5>P>0.2$; Frappell et al., 2001).

Inspiratory period, expiratory period and total breath time of burrowing owls breathing room air have not been previously reported. The mean duration of inspiration (T_I) in owls was 0.63± 0.09 s, while the mean expiratory period (T_E) was 0.91±0.22 s. The measured total breath time ($T_{TOT}=T_I+T_E$) was 1.54±0.28 s. There was no appreciable end-expiratory pause observed in owls during these experiments as typically occurs in mammals and other vertebrates (Frappell et al., 2001). The observed respiratory periods (T_I, T_E, and T_{TOT}) are nearly identical with and not different from ($0.9>P>0.5$) values predicted from allometry (Frappell et al., 2001).

The ratios of T_I and T_E to T_{TOT} tend to be interspecific constants in mammals ($T_I/T_{TOT}=0.35$; $T_E/T_{TOT}=0.65$) (Boggs and Tenney, 1984). However, these ratios are more variable in the birds studied to date. The T_I/T_{TOT} ratio in birds ranges from 0.36 in the pigeon (Williams et al., 1995) to 0.495 in the black-billed magpie (Boggs et al., 1997). The T_E/T_{TOT} ratio in birds is equally variable ranging from 0.51 to 0.64 (Williams et al., 1995; Boggs et al., 1997). The T_I/T_E ratio in burrowing owls and other birds is generally higher than it is in mammals. In the birds studied to date, the T_I/T_E ratios range from 0.56 in the pigeon (Williams et al., 1995) to 0.98 in the black-billed magpie (Boggs et al., 1997). It would appear that inspiration accounts for a greater fraction of total breath time in burrowing owls and other birds compared to mammals (Frappell et al., 2001). Despite the limited data available, it is clear that respiratory timing characteristics in birds are notably different from those of mammals and likely reflect differences between the two groups in mechanics and perhaps in respiratory drive (Frappell et al., 2001).

Mean values for arterial partial pressures of oxygen (PaO_2) and carbon dioxide ($PaCO_2$), and arterial pH (pH_a) obtained in the current study (Table 1) are similar to those reported previously (Maginniss and Kilgore, 1989; Kilgore et al., 1994) for burrowing owls breathing 21% O_2 ($P>0.50$ for PaO_2 and $PaCO_2$; $P>0.1$ for pH_a) and are not different from the blood gases of other birds breathing air (see Kilgore et al., 1994, for a review).

Mean arterial blood pressure (MABP) of owls breathing air obtained in this study (111±17 mm Hg) was nearly identical and not significantly different ($0.9>P>0.5$) from the pressures reported by Kilgore et al. (1994) (109±16 and 111±13 mm Hg). Also, the mean heart rate of burrowing owls breathing air in this study (269±29 beats min^{-1}) is not significantly different ($0.9>P>0.4$) from previously reported values (Kilgore et al., 1994) (284±67 and 260±22 beats min^{-1}) nor is it different ($0.4>P>0.2$) from what would be expected from allometry based on the mean body mass of the owls studied (307 beats min^{-1}) (Grubb, 1983).

The mean packed cell volume of the blood of burrowing owls in this study was 34.3±6.8% and comparable to previously reported values, 33.7±4.2% ($0.9>P>0.5$; Maginniss and Kilgore, 1989) and 36.6±2.5% ($0.4>P>0.2$; Boggs et al., 1983).

The mean oxygen convection requirement of burrowing owls breathing room air in this study (20.0±5.3) was significantly less ($0.02<P<0.05$) than the mean oxygen convection requirement (26.3±3.1) reported earlier (Boggs and Kilgore, 1983), the difference being attributable to the lower ventilation values, but similar oxygen consumption rates, recorded for owls in the present study. However, the present ratio is similar to and not significantly different ($0.9>P>0.5$) from the average convection requirement (22.7) for birds reported by Frappell et al. (2001).

4.2. Metabolic response of burrowing owls to hypoxia

Oxygen consumption rate of burrowing owls does not decline significantly with exposure to hypoxia ($P=0.452$; Table 1; Fig. 1B) despite a significantly reduced T_b in owls breathing 11% and 9% O_2 gas mixtures. This lack of a pronounced hypometabolic response to hypoxia in owls, which was also observed in our earlier study (Boggs and Kilgore,

1983) where owls were exposed to mild levels of hypoxia, represents an exception to the common vertebrate response (Wood, 1991; Mortola, 1993). All small to medium sized adult and newborn mammals studied to date show a decline in metabolism while acutely breathing gas mixtures containing only 10% O_2 (Frappell et al., 1992; Mortola, 1993; Mortola and Gautier, 1995; Walsh et al., 1996). Even in large mammals a hypoxic hypometabolic response is observed, but their response is reduced in magnitude as a result of their low mass-specific metabolic rates (Mortola, 1993). In birds, the pattern is less clear. A few species have been shown to have a marked hypometabolic response to hypoxia below an inspired PO_2 of 80 mm Hg (Weathers and Snyder, 1974; Castro et al., 1985). However, other studies of small passerines have been unable to demonstrate a hypometabolic response even at simulated high altitudes of up to 10 km and at low experimental temperatures that elevate thermogenic requirements (Tucker, 1968; Clemens, 1988; Novoa et al., 1991). Larger birds are equally variable in their metabolic response to hypoxia. Pekin ducks may or may not show a marked hypometabolism at simulated altitudes above 9 km (Colacino et al., 1977; Black and Tenney, 1980), while bar-headed geese show an *increase* in oxygen consumption even when exposed to simulated altitudes in excess of 11 km (Black and Tenney, 1980)!

Whether or not the burrowing owls in the current study would have shown a significant hypometabolic response to extreme hypoxia with a more prolonged exposure is unknown, although the 15–20 min exposure time used in this study is generally sufficient for oxygen consumption to be significantly depressed in 100–150 g quail (R.W. Bavis; unpublished observation). A time-dependent drop in metabolism has been demonstrated in rats (see for example, Ling et al., 1996). In their study, untreated control rats did not show a hypoxic hypometabolic response to acute hypoxia ($PIO_2 \sim 84$ mm Hg) during the first 20 min of exposure, but metabolism declined significantly between 20 and 30 min of exposure.

The pronounced hypometabolism in adult vertebrates exposed to hypoxia results from the inhibitory effects of hypoxia on hypothalamic thermoregulatory centers, the direct depression of cellular metabolism by hypoxia and from Arrhenius (Q_{10}) effects of a decline in T_b on metabolism (Mortola, 1993; Frappell et al., 1995; Mortola and Gautier, 1995). A reduction in metabolism is thought to be protective during acute exposure to hypoxia, limiting oxygen demand when oxygen levels in the inspired air are reduced (Wood, 1991; Mortola, 1993).

The maintenance of a normal oxygen consumption in the face of hypoxia suggests that burrowing owls can maintain oxygen delivery to their tissues despite the low PO_2 in the inspired air. Clearly the significantly elevated oxygen convection requirement of owls exposed to 11% and 9% O_2 (Fig. 1C) contributes to this oxygen delivery by increasing the oxygen content of the blood. This improvement in oxygenation, coupled with other cardiopulmonary features of burrowing owls, for example, their elevated heart rate with exposure to hypoxia and their larger than expected heart size and presumably larger cardiac output, may improve oxygen delivery and thereby counter the need for a reduced metabolism (Boggs et al., 1983).

4.3. Hypoxic ventilatory threshold of burrowing owls

The brisk ventilatory response observed in burrowing owls exposed to extreme levels of hypoxia in this study, is consistent with what has been observed in other birds (see Colby et al., 1987 for a review).

How does the HVT of burrowing owls compare to that of other birds? The hypoxic ventilatory threshold, defined in terms of PaO_2, has only been measured in a few bird species (Table 2). HVT in these species varies from 38 mm Hg in the bar-headed goose (Black and Tenney, 1980) to 70 mm Hg in the ring-necked pheasant (Boggs and Birchard, 1983) and Pekin duck (Black and Tenney, 1980). Variation in the HVT of these birds can be explained in terms of the hemoglobin O_2 affinity of their blood. As in other vertebrates, there is a parsimonious relationship between HVT and the PaO_2 at which the species' hemoglobin is 60–80% saturated (van Nice et al., 1980; Boggs, 1995). The same is true of burrowing owls. Based on the HVT of burrowing owls determined in this study, 58 mm Hg, saturation of the owl's hemoglobin would equal 73% (Maginniss and Kilgore, 1989). This relationship between HVT and the position of the oxygen equilibrium curve suggests that ventilation is increased only after a sufficient desaturation of the hemoglobin has occurred. The increased ventilation, leads to an elevated partial pressure of oxygen at the exchange surface and an improved oxygen transport (Hopkins and Powell, 2001).

In both our earlier study (Boggs and Kilgore, 1983) and the present study, we failed to detect a significant increase in ventilation or oxygen convection requirement in owls breathing 13% O_2, even though owls in the current study exposed to 13% O_2 had a mean PaO_2 (54 mm Hg) slightly below the HVT (58 mm Hg) and a significantly lowered $PaCO_2$. This result is not surprising. Total ventilation measurements are quite variable in awake unrestrained animals, particularly at moderate levels of hypoxia near the HVT. Given the inter-individual variation in ventilation and variation in PaO_2 at a given FIO_2, not all individuals are at HVT while breathing 13% O_2. The mean increase in ventilation observed in owls in the present study breathing 13% O_2 was 22.8%, but the standard deviation of the sample was 29.5%. Three of the 6 owls at this treatment show little or no increase in ventilation while breathing 13% O_2; one actually had a total ventilation slightly below its normoxic value. Given this variability, a larger sample size would be required to achieve statistical significance at $\alpha = 0.05$ (we estimate this number to be 16). In view of the inherent difficulty in precisely determining HVT, piecewise regression is a valuable technique (Toms and Lesperance, 2003). A piecewise regression model consisting of two straight lines joined at a break point fits the data well and is biologically meaningful (Neter and Wasserman, 1974).

Because of the hypometabolic response shown by many species exposed to hypoxia, the oxygen convection requirement might be a better measure of respiratory responsiveness to

hypoxia (Boggs, 1995; Tattersall et al., 2002). To that end, we also determined the PaO_2 of burrowing owls at which there was a significant increase in oxygen convection requirement (Fig. 6B). Because oxygen consumption rates in owls are unaffected by hypoxia (Fig. 1B) the hypoxic ventilatory threshold expressed as percent change in oxygen convection requirement (Fig. 6B) is identical (58 mm Hg) to that determined from piecewise regression of percent change in ventilation.

4.4. Relationship between hypoxic ventilatory threshold and hemoglobin oxygen affinity in birds

Despite the few data available, it is possible to analyze the relationship between HVT and hemoglobin O_2 affinity in birds. Data from the literature on HVT and P_{50} (a readily available index of the position of the oxygen equilibrium curve and hemoglobin O_2 affinity) included in the regression analyses (traditional and phylogenetically corrected) are shown in Table 2. HVT and P_{50} are linearly related in birds as is apparent in Fig. 7. A strong positive relationship between HVT and P_{50} is also evident in other vertebrates (Boggs, 1995). Traditional regression of HVT on P_{50} explains a significant ($P=0.025$) portion of the variance in HVT of birds. The coefficient of determination (R^2) of the relationship is 0.76. The slope of the regression line (1.53) is significantly greater than zero ($P=0.025$).

Because of common ancestry of taxa, species are not independent variates. Therefore, it was desirable to analyze the HVT *vs.* P_{50} relationship by calculating phylogenetically independent contrasts, which circumvent the non-independence problem in traditional linear regression. Analysis of the HVT *vs.* P_{50} relationship with phylogenetically independent contrasts yielded similar results to traditional regression. The slope considering degree of relatedness is 1.86 (Fig. 7) and the R^2 of the relationship is 0.84. The slopes of the two predictive equations are not statistically different ($0.9>P>0.5$). If anything, correcting for phylogeny makes the relationship between HVT and hemoglobin O_2 affinity more robust and convincing. Numerous other comparative studies of birds and mammals where both non-phylogenetic statistics and phylogenetically independent contrasts have been calculated have yielded similar slopes and intercepts of the same relationships (Ricklefs and Starck, 1996; Frappell et al., 2001; Hempleman et al., 2005).

The HVT of burrowing owls, defined in terms of PaO_2, is consistent with expectations based on P_{50} and falls well within the 95% prediction limits of the expected relationship for birds (Fig. 7). Thus, as we hypothesized earlier, owls are not exceptional in this regard despite their burrowing lifestyle.

4.5. Possible mechanism behind the HVT vs. P_{50} relationship

The strong positive relationship between hypoxic ventilatory threshold and hemoglobin O_2 affinity of birds established in this study is similar to that observed in mammals (Boggs, 1995; Boggs et al., 1998; Boggs et al., 1999; Frappell et al., 2002). However, the mechanism responsible for the stimulation of ventilation at a partial pressure of oxygen at which hemoglobin becomes sufficiently desaturated ($SaO_2=0.6$ to 0.85) to alter oxygen content of the arterial blood is still a matter of conjecture (Peers and Kemp, 2001). Early suggestions that oxygen-sensing molecules involved in O_2-transduction in the carotid bodies react to hypoxic stimuli in parallel with hemoglobin, thereby linking hypoxic ventilatory threshold and hemoglobin O_2 affinity, remain plausible (Boggs, 1995; Boggs et al., 1999; Peers and Kemp, 2001). Recent reports of O_2-sensory mechanisms in carotid bodies include heme-containing enzymes (e.g., hemeoxygenase-2, cyctochrome *c* oxidase, NADPH oxidase) that may be excellent candidates for the matching of hypoxic ventilatory threshold to hemoglobin O_2 affinity (Carroll and Kim, 2005; Kemp, 2005; Donnelly and Carroll, 2005; Ramirez et al., 2007).

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