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Control of breathing and adaptation to high altitude in the bar-headed goose

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Scott GR, Milsom WK. Control of breathing and adaptation to high altitude in the bar-headed goose. *Am J Physiol Regul Integr Comp Physiol* 293: R379–R391, 2007. First published May 9, 2007; doi:10.1152/ajpregu.00161.2007.—The bar-headed goose flies over the Himalayan mountains on its migratory route between South and Central Asia, reaching altitudes of up to 9,000 m. We compared control of breathing in this species with that of low-altitude waterfowl by exposing birds to step decreases in inspired O_2 under both poikilocapnic and isocapnic conditions. Bar-headed geese breathed substantially more than both greylag geese and pekin ducks during severe environmental (poikilocapnic) hypoxia (5% inspired O_2). This was entirely due to an enhanced tidal volume response to hypoxia, which would have further improved parabronchial (effective) ventilation. Consequently, O_2 loading into the blood and arterial P_{O_2} were substantially improved. Because air convection requirements were similar between species at 5% inspired O_2, it was the enhanced tidal volume response (not total ventilation per se) that improved O_2 loading in bar-headed geese. Other observations suggest that bar-headed geese depress metabolism less than low-altitude birds during hypoxia and also may be capable of generating higher inspiratory airflows. There were no differences between species in ventilatory sensitivities to isocapnic hypoxia, the hypoxia-induced changes in blood CO_2 tensions or pH, or hypercapnic ventilatory sensitivities. Overall, our results suggest that evolutionary changes in the respiratory control system of bar-headed geese enhance O_2 loading into the blood and may contribute to this species' exceptional ability to fly high.

hypoxic ventilatory response; birds; respiration; metabolism; carbon dioxide

TO MAINTAIN PHYSIOLOGICAL FUNCTION at high altitude, cellular O_2 supply and demand must remain matched, even though environmental O_2 availability is reduced. Substantial reductions in O_2 demand are not possible in animals that remain active in hypoxia, however, so the capacity to transport O_2 must be increased in high-altitude species. Oxygen transport occurs along the pathway from air to mitochondria, which can be conceptually separated into four steps: ventilation, pulmonary diffusion, circulation (perfusion), and tissue diffusion (49, 59). In animals that adapt (in an evolutionary sense) to high altitude, natural selection could act at any step of the O_2 pathway. Physiological theories for O_2 transport predict that either every step in the O_2 pathway should be subject to selection equally (the symmorphosis concept) (60) or selection could act disproportionately at only one or a few steps in the pathway (the control coefficients concept) (34).

As the first step in the O_2 transport pathway, breathing is responsible for gas transfer between the lungs and the environment. Exposure to environmental hypoxia causes an immediate increase in breathing due to stimulation of arterial chemoreceptors, followed by many subsequent time-dependent modifications that are thought to originate from multiple sites in the reflex pathway (46). In addition to these modifications, changes in metabolic state and differences in pulmonary mechanics will also influence breathing.

Evolutionary changes in ventilatory control of high altitude-adapted species are poorly documented. Multiple human populations have adapted to different high-altitude regions of the world, and although it is clear that evolutionary changes in respiratory control exist in these groups, different adaptations have arisen in each: high-altitude Quechuans from the Andean plateau have an inherently reduced hypoxic ventilatory response (HVR) compared with low-altitude residents (8), whereas high-altitude Tibetans have an enhanced HVR (63). Little is known about how control of breathing has evolved in other vertebrates during high-altitude adaptation.

Birds in general have excellent hypoxia tolerance and can maintain normal function during hypoxia too severe for most mammals to survive (55). Some species of birds are exceptional in this regard. The bar-headed goose (*Anser indicus*) flies over the Himalayan mountains twice a year on its migratory route between its wintering grounds in southern Asia and its summering grounds on the Tibetan plateau. This species has been repeatedly observed flying above the highest peaks in the range (35, 53), where oxygen levels can be five times lower than those at sea level. This feat is especially impressive considering that the bar-headed goose increases its rate of O_2 consumption 10- to 20-fold above resting levels during flight (57).

Several previous physiological studies have sought to determine how bar-headed geese can sustain the aerobic requirements of flight at such high altitudes (reviewed in Refs. 17, 49). Hemoglobin of this species has been shown to have an inherently higher O_2 affinity, which is almost entirely due to a single amino acid point mutation in the α_A polypeptide chain (44, 58, 64). This mutation is adaptive in bar-headed geese and facilitates O_2 loading during hypoxia. Although many other physiological attributes of birds in general may preadapt this species for high-altitude flight, no other true adaptations have yet been found in bar-headed geese (3, 4, 18–20, 22, 23, 47, 56).

In a recent theoretical study (49), we found that a heightened ability to increase ventilation should enhance O_2 transport substantially during hypoxia in birds. On the basis of these findings, we hypothesized that high-altitude adaptation in bar-headed geese would involve changes in ventilatory control such that this species would have an enhanced HVR (i.e., it would breathe more) and load more O_2 into the blood during hypoxia. To test this hypothesis, we compared the control of breathing in bar-headed geese and two other waterfowl species: the greylag goose (*Anser anser*), which is a close relative of the

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bar-headed goose, and the pekin duck (*Anas platyrhynchos*), which is a more distant relative (pekin ducks are a domesticated subspecies of mallard ducks). These species are thought to migrate primarily at low to moderate altitudes (<4,000 m), although there have been occasional sightings at higher elevations (40, 52, 53). We predicted that adaptive differences in control of breathing would exist between bar-headed geese and both low-altitude species, which, because of the evolutionary relationship between the species selected, could not be due to neutral phylogenetic effects.

MATERIALS AND METHODS

Experimental animals. Experiments were performed on 8 bar-headed geese (1.9–2.6 kg), 6 greylag geese (3.2–4.9 kg), and 10 pekin ducks (2.3–3.7 kg). All animals were bred and raised at sea level, either at the Animal Care Facility of the University of British Columbia (UBC) or by local suppliers. Animals were housed outdoors at UBC and had no prior exposure to high altitude. Birds were starved for 1 day before each experiment but continued to receive unlimited access to water. All animal care and experimentation were conducted according to UBC animal care protocol no. A04-1013.

Surgical procedures. All birds underwent surgery 1–2 days before experimentation. Birds were given an intramuscular injection of telazol (Wyeth; 20–25 mg/kg) to induce moderate anesthesia and were lightly restrained in dorsal recumbency. Animals were maintained under this general anesthesia with one to two additional injections of telazol (8–10 mg/kg) as needed. Flexible polyethylene cannulas (PE-90) filled with 1,000 IU/ml heparinized saline were implanted under local anesthesia (Lidocaine; Langford; administered subcutaneously) in the right brachial artery and vein (lateral to the humerus) and were slowly advanced ~8 cm. The clavicular air sac was intubated with a small tube (7 mm in diameter and ~10 mm long) containing a rubber plug under local anesthesia. The tube was secured to the skin and underlying air sac membrane with suture. On the day of the experiment, the rubber plug was removed and replaced with a similar plug that allowed the passage of a heat-flared PE-90 cannula such that gas samples could be drawn continuously from the clavicular air sac.

Measurements. Body plethysmography was used to measure ventilation, as previously described (13, 14). Briefly, the plethysmograph consisted of two parts, a body compartment and a head compartment, separated from each other by a flexible latex collar. The head compartment was used to administer specific gas mixtures for the animals to breathe. The composition of dry gas flowing into the head compartment was controlled by mixing N_2, O_2, and CO_2 through a series of calibrated flowmeters and was monitored with O_2 (Raytech) and CO_2 analyzers (Beckman LB-2; hypercapnic hypoxia exposure only). Gas was humidified before entering the head compartment, and the flow rate was never < 15 l/min.

Changes in body volume (due to ventilatory movements) in the body compartment were detected with a pneumotachograph (Fleisch) connected to a differential pressure transducer (Validyne), which was calibrated to yield a measurement of ventilatory flow. Arterial blood pressure was continuously monitored using a physiological pressure transducer (Narco Scientific) connected to the brachial artery cannula. Gas was continuously drawn from the clavicular air sac (poikilocapnic and isocapnic hypoxia exposures only) at a rate of ~100 ml/min, and its fractional CO_2 composition ($F_{CL_{CO_2}}$) was measured with a CO_2 analyzer (Beckman). Ventilatory flow, fractional O_2 composition of gas entering and leaving the head compartment, airflow through the head compartment, and $F_{CL_{CO_2}}$ were recorded to a computer at a 125-Hz sampling frequency per channel using Windaq data acquisition software (Dataq Instruments).

After being drawn, arterial and mixed venous blood samples (0.7 ml) were immediately used to measure blood gases, pH, and O_2 concentration. Arterial and venous O_2 (P_{O_2}) and CO_2 (P_{CO_2}) tensions and arterial and venous pH were determined using Radiometer blood gas/pH electrodes maintained at avian core body temperature (41°C). The electrodes were calibrated before each sample using saturated gases and commercially prepared pH buffers (VWR). Arterial and venous O_2 concentrations were determined at avian body temperature using the method of Tucker (54). The bicarbonate ion concentrations of blood were calculated using the Henderson-Hasselbach equation, assuming a pK of 6.090 and a solubility coefficient of 0.0282 mM/Torr in plasma (31).

Experimental protocol. Birds were placed in the experimental apparatus and allowed 60–90 min to adjust to their surroundings. The birds were then exposed to progressive step reductions in the fractional O_2 composition of inspired gas ($F_{I_{O_2}}$; in declining order, 0.209, 0.12, 0.09, 0.07, 0.05, and, in bar-headed geese only, 0.04). This hypoxia exposure was performed twice, once under poikilocapnic conditions and once under isocapnic conditions. The poikilocapnic protocol was performed first in half of the experiments, and the isocapnic protocol was performed first in the other half, such that the effects of time-dependent processes between poikilocapnic and isocapnic data were minimized. For poikilocapnic hypoxia exposure, $F_{I_{O_2}}$ was reduced as described above and the inspired CO_2 fraction ($F_{I_{CO_2}}$) was zero. Because $F_{I_{CO_2}}$ was not manipulated, blood CO_2 levels (and thus $F_{CL_{CO_2}}$) fell proportionately with the hypoxia-induced hyperventilation. During isocapnic hypoxia exposure, $F_{I_{CO_2}}$ was increased slowly as necessary while $F_{CL_{CO_2}}$ was monitored to maintain blood CO_2 levels constant, and $F_{I_{O_2}}$ was reduced in the same manner as for poikilocapnic hypoxia. After 15 min of exposure to each $F_{I_{O_2}}$ level, arterial and venous blood samples were withdrawn slowly and analyzed as described above. As much of the sampled blood as possible was returned to the bird. Birds were exposed to each $F_{I_{O_2}}$ level for 25 min. Each bird was allowed ~90 min of recovery between the first and second hypoxia exposures, at which time respiratory variables and $F_{CL_{CO_2}}$ had returned to resting levels. After both hypoxia exposures were complete, birds recovered for 15 min (at $F_{I_{O_2}} = 0.12$, $F_{I_{CO_2}} = 0$) and were then exposed to hypercapnia ($F_{I_{O_2}} = 0.12$, $F_{I_{CO_2}} = 0.05$) for 25 min, with blood samples being drawn at 15 min as before.

Data and statistical analyses. All data acquired in Windaq were analyzed using a specially written Matlab (version 7, MathWorks) program. Average values were calculated for each variable during the interval between 10 and 20 min of exposure to each inspired gas level. Inspiratory tidal volume was determined by integrating positive periods of ventilatory flow. Total ventilation was calculated from the product of tidal volume and breathing frequency. The rate of O_2 consumption was calculated from the product of airflow through the head compartment and the F_{O_2} difference between the compartment's inflow and outflow (i.e., the respiratory quotient, RQ, was assumed to be 1). Although RQ values in birds typically range from <0.7 to 1.0, and our calculations ignore this potential source of variation, we used the same value in all cases, so this will not alter our results. Because water vapor was removed from this gas before analysis, the rates of O_2 consumption were determined as described by Withers (62). Air convection requirement was calculated as the quotient of total ventilation and the rate of O_2 consumption. Average values for inspiratory time (T_I), expiratory time (T_E), peak inspiratory flow rate, peak expiratory flow rate, and $F_{CL_{CO_2}}$ were also determined for each minute analyzed.

Data are means ± SE. Within each species, all data in each experiment were analyzed using repeated-measures analysis of variance, with Holm-Sidak post hoc tests. Comparisons between species were made for respiratory and blood gas data using two-factor (species and $F_{I_{O_2}}$) repeated-measures analysis of variance and Holm-Sidak post hoc tests within each $F_{I_{O_2}}$. Linear regression was used to assess the relationships between total ventilation and peak inspiratory/expiratory flow rates. Statistical tests were performed using SigmaStat

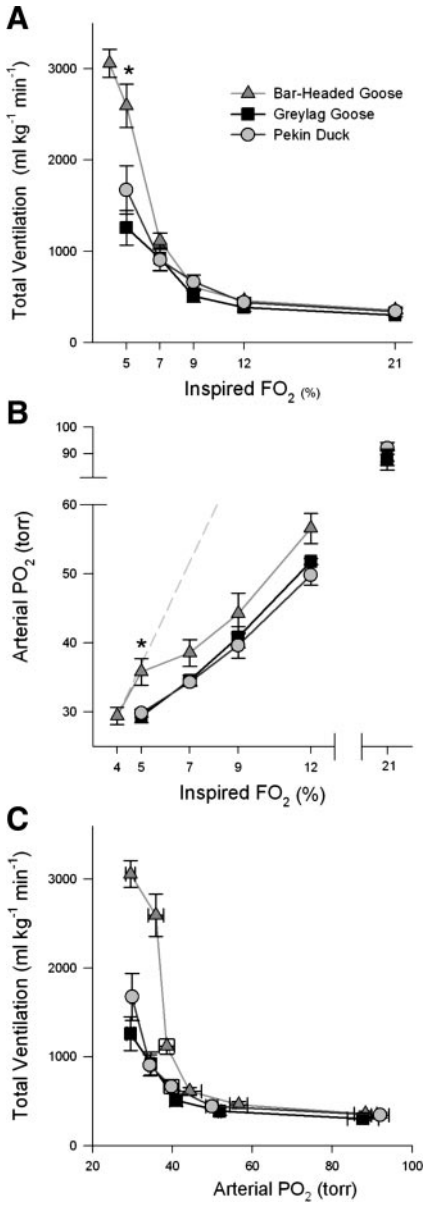

Fig. 1. Total ventilation (*A*) was higher in bar-headed geese during severe poikilocapnic (uncontrolled CO_2) hypoxia than in both greylag geese and pekin ducks. This resulted in higher arterial O_2 tensions (P_{O_2}; *B*) during reduced inspired O_2 fraction (F_{O_2}). The shaded dashed line in *B* represents the P_{O_2} of inspired air. The response of total ventilation to reductions in arterial P_{O_2} (*C*) was greater in bar-headed geese. Data are means ± SE. For each species, total ventilation increased significantly and arterial P_{O_2} decreased significantly after each step reduction of inspired F_{O_2} ($P < 0.05$). *$P < 0.05$, significant difference between bar-headed geese and both low-altitude species.

software (version 3.0; Systat Software). A significance level of $P < 0.05$ was used throughout.

RESULTS

Poikilocapnic hypoxic ventilatory responses. During the poikilocapnic hypoxia experiment, each progressive step reduction in $F_{I_{O_2}}$ increased total ventilation and reduced arterial O_2 tension (Pa_{O_2}) for all three species (Fig. 1). The ventilatory response was significantly greater in bar-headed geese: this species increased total ventilation by 7.2-fold above normoxic levels at 5% $F_{I_{O_2}}$, compared with only 4.2-fold in greylag geese and 4.9-fold in pekin ducks (Fig. 1*A*). Pa_{O_2} was generally higher (4–7 Torr) in bar-headed geese at all levels of hypoxia, and this was significant at 5% $F_{I_{O_2}}$ (by 6 Torr). Only bar-headed geese could tolerate 4% $F_{I_{O_2}}$, which resulted in an 8.6-fold increase in ventilation above normoxic levels. Interestingly, at both 5 and 4% $F_{I_{O_2}}$, Pa_{O_2} in bar-headed geese was approximately equal to inspired P_{O_2} (the theoretical maximum, shaded dashed line in Fig. 1*B*); Pa_{O_2} never reached inspired levels in either greylag geese or pekin ducks. The enhanced ventilatory response to poikilocapnic hypoxia in bar-headed geese is apparent in the relationship between ventilation and Pa_{O_2} (Fig. 1*C*).

The higher total ventilation in bar-headed geese was caused by an enhanced tidal volume response to poikilocapnic hypoxia (Fig. 2). Tidal volume was significantly higher in bar-headed geese than in both other species at 9, 7, and 5% $F_{I_{O_2}}$, but breathing frequency was generally similar. As a result, for any level of total ventilation, bar-headed geese had a higher tidal volume and lower breathing frequency (right-shifted curve in Fig. 2). The ventilatory response to poikilocapnic hypoxia involved reductions in T_I and T_E in all three species, and T_E tended to fall more such that the ratio of T_I/T_E increased (Table 1).

In concert with progressive reductions in Pa_{O_2} during poikilocapnic hypoxia were declines in arterial O_2 concentration (Ca_{O_2}), venous O_2 tension (Pv_{O_2}), and venous O_2 concentration (Cv_{O_2}) (Table 2). Bar-headed geese maintained a higher Ca_{O_2} than greylag geese and pekin ducks at 5% $F_{I_{O_2}}$, consistent with the observed Pa_{O_2} differences between species. When Ca_{O_2} was expressed relative to Pa_{O_2} and the data were fitted with a sigmoidal Hill equation {i.e., $Ca_{O_2} = (Pa_{O_2})^{n_H}/[(P_{50})^{n_H} + (Pa_{O_2})^{n_H}]$}, bar-headed geese, greylag geese, and pekin ducks had in vivo values of P_{50} (the P_{O_2} causing 50% saturation of blood O_2 binding capacity) of 35.4, 32.7, and 35.8 Torr, respectively.

Isocapnic hypoxic ventilatory responses. As during poikilocapnic hypoxia (when CO_2 levels in the blood were allowed to fall), during isocapnic hypoxia (when CO_2 levels in the blood

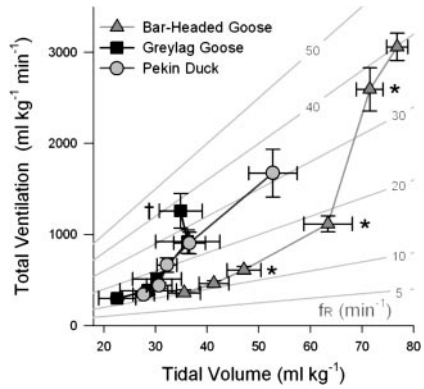

Fig. 2. Ventilatory response of bar-headed geese to poikilocapnic (uncontrolled CO_2) hypoxia involved a different breathing pattern from that of both greylag geese and pekin ducks: for any total ventilation, bar-headed geese had a higher tidal volume and lower breathing frequency (right-shifted curve). Shaded lines represent breathing frequency (f_R) isopleths. *$P < 0.05$, significant difference in tidal volume between bar-headed geese and both low-altitude species. †$P < 0.05$, significant difference in tidal volume between low-altitude species.

Table 1. *Respiratory variables during poikilocapnic hypoxia*

$F_{I_{O_2}}$	T_I	T_E	T_I/T_E	$P_{CL_{CO_2}}$
		Bar-headed goose		
21%O_2	2.32±0.13[a]	3.73±0.25[a]	0.64±0.05[a]	41.5±2.4[a]
12%O_2	2.21±0.11[a]	3.26±0.27[a]	0.70±0.03[a]	31.3±2.3[b]
9%O_2	1.99±0.09[b]	2.72±0.24[a]	0.76±0.04[b]	23.1±1.7[c]
7%O_2	1.61±0.05[c]	1.83±0.10[b]	0.89±0.04[c]	16.0±1.1[d]
5%O_2	0.84±0.11[d]	0.93±0.12[c]	0.90±0.02[c]	7.9±1.1[c]
4%O_2	0.73±0.05[d]	0.79±0.04[c]	0.92±0.05[c]	6.1±1.3[f]
		Greylag goose		
21%O_2	2.02±0.28[a]	2.43±0.40[a]	0.86±0.07[ab]	39.8±3.2[a]
12%O_2	1.82±0.27[a]	2.61±0.43[a]	0.72±0.05[a]	29.6±2.4[b]
9%O_2	1.56±0.12[a]	2.04±0.24[ab]	0.79±0.07[ab]	22.2±1.5[c]
7%O_2	1.13±0.09[b]	1.26±0.17[b]	0.93±0.07[b]	15.9±0.6[d]
5%O_2	0.88±0.13[b]	0.83±0.04[c]	1.06±0.11[b]	10.1±0.8[e]
		Pekin duck		
21%O_2	1.92±0.22[a]	3.06±0.26[a]	0.65±0.10[a]	31.9±2.4[a]
12%O_2	1.55±0.12[a]	2.72±0.26[a]	0.58±0.04[a]	25.6±1.7[b]
9%O_2	1.27±0.10[a]	1.87±0.24[a]	0.71±0.05[a]	18.5±1.3[c]
7%O_2	1.10±0.10[b]	1.49±0.20[b]	0.77±0.04[a]	13.3±1.0[d]
5%O_2	0.98±0.10[b]	1.12±0.15[c]	0.89±0.03[b]	7.7±1.1[e]

$F_{I_{O_2}}$, inspired O_2 fraction (%); T_I, inspiratory time (s); T_E, expiratory time (s); $P_{CL_{CO_2}}$, clavicular air sac CO_2 tension (Torr). Statistics were performed within each species only. [a,b,c,d,e,f]$P < 0.05$, for each parameter, treatments with different letters are significantly different.

were maintained) each reduction in $F_{I_{O_2}}$ normally increased total ventilation and reduced Pa_{O_2} (Fig. 3). The only exception was that total ventilation did not increase during exposure to the lowest $F_{I_{O_2}}$ level studied. In general, total ventilation and Pa_{O_2} were higher in isocapnic hypoxia than in poikilocapnic hypoxia in all three species. However, the ventilatory response of bar-headed geese to isocapnic hypoxia was not enhanced compared with that of pekin ducks: both species increased total ventilation by approximately sevenfold above normoxic levels at 5% $F_{I_{O_2}}$ (Fig. 3*A*). Greylag geese had lower total ventilation than both other species at 7 and 5% $F_{I_{O_2}}$ and could only increase total ventilation 4- to 4.5-fold above normoxic levels. Pa_{O_2} was similar in all species at all levels of $F_{I_{O_2}}$, and it approached inspired P_{O_2} at 7% $F_{I_{O_2}}$ and below (Fig. 3*B*).

Tidal volumes were generally higher during isocapnic hypoxia (Fig. 4) than during poikilocapnic hypoxia (Fig. 2). Furthermore, and in contrast to poikilocapnic hypoxia, there were few differences between species in tidal volume responses to isocapnic hypoxia. Tidal volume was reduced in greylag geese at 9, 7, and 5% $F_{I_{O_2}}$, but otherwise all species responses were similar for both tidal volume and breathing frequency.

The ventilatory response to isocapnic hypoxia involved reductions in T_I and T_E and an increase in T_I/T_E (Table 3). Ca_{O_2}, Pv_{O_2}, and Cv_{O_2} (Table 4) all decreased progressively. For both poikilocapnic and isocapnic hypoxia in all species, Cv_{O_2} was almost always near zero for the lowest $F_{I_{O_2}}$ level studied; however, this tended to occur in conjunction with lower Pv_{O_2} levels in bar-headed geese. When Ca_{O_2} was expressed relative to Pa_{O_2} and fitted with a Hill equation as for poikilocapnic hypoxia, bar-headed geese, greylag geese, and pekin ducks had in vivo P_{50} values of 38.2, 45.6, and 48.1 Torr, respectively.

Acid-base regulation during hypoxia. The increases in ventilation during poikilocapnic hypoxia caused progressive reductions in the CO_2 tension of arterial blood (Pa_{CO_2}) (Fig. 5*A*), venous blood (Pv_{CO_2}) (Table 2), and the respiratory system (clavicular air sac, $P_{CL_{CO_2}}$) (Table 1) in all three species. This respiratory hypocapnia did not cause any significant arterial pH disturbance in any species (Fig. 5*A*), and this may have been partly due to progressive reductions in arterial bicarbonate concentrations (assuming calculated $[HCO_3^-]$ is representative of actual $[HCO_3^-]$). There were statistically insignificant trends suggestive of a respiratory alkalosis during moderate hypoxia, followed by metabolic acidosis at more severe levels of hypoxia in all species.

Table 2. *Blood gas variables during poikilocapnic hypoxia*

$F_{I_{O_2}}$	Ca_{O_2}	Pv_{O_2}	Pv_{CO_2}	pHv	$[HCO_3^-]v$	Cv_{O_2}
			Bar-headed goose			
21%O_2	4.57±0.20[a]	40.9±4.3[a]	32.6±1.3[a]	7.36±0.04[a]	17.0±1.3[a]	2.60±0.37[a]
12%O_2	4.54±0.30[a]	31.0±2.4[ab]	28.3±1.8[ab]	7.33±0.08[a]	14.3±2.1[b]	2.27±0.29[a]
9%O_2	3.47±0.36[b]	24.2±1.6[bc]	24.6±1.5[bc]	7.37±0.08[a]	13.7±1.8[b]	1.15±0.30[b]
7%O_2	3.18±0.31[c]	22.3±1.7[c]	19.4±1.5[cd]	7.36±0.11[a]	11.4±2.8[c]	1.10±0.22[b]
5%O_2	2.31±0.20[d]	16.5±1.7[c]	14.4±1.4[de]	7.23±0.12[a]	6.4±1.9[d]	0.69±0.30[bc]
4%O_2	1.10±0.13[e]	16.6±4.3[c]	11.0±2.0[e]	7.26±0.11[a]	5.3±2.4[d]	0.16±0.07[c]
			Greylag goose			
21%O_2	4.96±0.15[a]	55.8±3.8[a]	31.3±1.3[a]	7.37±0.06[a]	17.6±3.8[a]	3.23±0.30[a]
12%O_2	4.60±0.16[b]	38.1±1.4[b]	27.2±1.4[ab]	7.35±0.07[a]	14.4±2.6[b]	2.85±0.12[a]
9%O_2	4.02±0.14[c]	31.8±0.9[bc]	21.7±0.8[b]	7.32±0.10[a]	11.0±2.9[c]	1.86±0.25[b]
7%O_2	2.95±0.29[d]	25.8±2.7[c]	19.3±1.2[bc]	7.36±0.04[a]	10.1±1.2[d]	1.28±0.26[b]
5%O_2	1.54±0.22[e]	21.6±3.0[c]	15.7±1.2[c]	7.19±0.10[a]	5.0±2.4[e]	0.38±0.15[c]
			Pekin duck			
21%O_2	4.83±0.32[a]	60.8±1.1[a]	34.2±1.7[a]	7.41±0.06[a]	20.3±3.1[a]	3.40±0.25[a]
12%O_2	3.83±0.29[b]	36.8±1.8[ab]	27.5±1.4[b]	7.44±0.03[a]	17.1±2.5[b]	2.22±0.25[b]
9%O_2	2.94±0.25[c]	28.7±1.2[bc]	22.4±1.2[c]	7.34±0.11[a]	11.8±2.1[c]	1.43±0.24[c]
7%O_2	2.28±0.21[d]	24.4±1.2[c]	18.7±1.1[c]	7.32±0.14[a]	10.1±1.9[d]	1.23±0.14[c]
5%O_2	1.97±0.11[e]	21.5±0.7[c]	20.8±5.6[c]	7.23±0.18[a]	6.7±2.2[e]	0.89±0.10[d]

$F_{I_{O_2}}$, inspired O_2 fraction (%); Ca_{O_2}, arterial O_2 content (mM); Pv_{O_2}, venous O_2 tension (Torr); Pv_{CO_2}, venous CO_2 tension (Torr); pHv, venous pH; $[HCO_3^-]v$, venous bicarbonate concentration (mM); Cv_{O_2}, venous O_2 content (mM). Statistics were performed within each species only. [a,b,c,d,e,f]$P < 0.05$, for each parameter, treatments with different letters are significantly different.

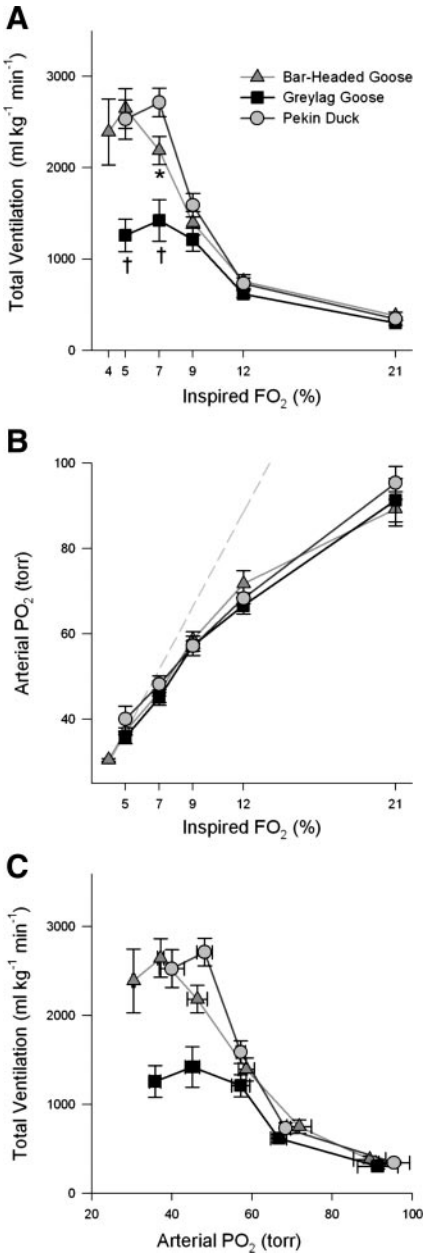

Fig. 3. Total ventilation (*A*) was not higher in bar-headed geese than in pekin ducks during isocapnic (constant arterial CO_2) hypoxia, but greylag geese had a reduced ventilatory response to isocapnic hypoxia. There were no differences in arterial P_{O_2} (*B*) at any level of inspired F_{O_2}. The shaded dashed line in *B* represents the P_{O_2} of inspired air. CO_2 was added to inspired gas as necessary to maintain isocapnia. Data are means ± SE. Total ventilation increased in each species after each step reduction of inspired F_{O_2} until 7% (but not at 5%) in greylag geese and pekin ducks and until 5% (but not at 4%) in bar-headed geese ($P < 0.05$). Arterial P_{O_2} in each species decreased significantly after each step reduction of inspired F_{O_2} ($P < 0.05$). *$P < 0.05$, significant difference between bar-headed geese and both low-altitude species. †$P < 0.05$, significant difference between low-altitude species.

Because CO_2 was added to inspired gas during isocapnic hypoxia, Pa_{CO_2} remained constant in all species (Fig. 5*B*), and Pv_{CO_2} (Table 4) and $P_{CL_{CO_2}}$ (Table 3) experienced only small, inconsistent changes. Arterial pH homeostasis was therefore maintained during moderate hypoxia. During more severe hypoxia, all species experienced an isocapnic metabolic acidosis of arterial blood, in conjunction with reductions in arterial bicarbonate. For both poikilocapnic and isocapnic hypoxia experiments, venous pH and bicarbonate levels changed in a similar manner to those in arterial blood (Tables 2 and 4).

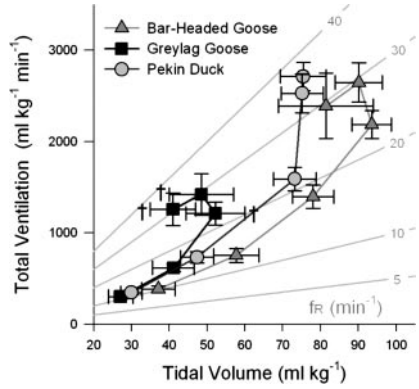

Fig. 4. Change in breathing pattern during isocapnic (constant arterial CO_2) hypoxia was similar in bar-headed geese, greylag geese, and pekin ducks, except in greylag geese during severe hypoxia. Shaded lines represent f_R isopleths. Data are means ± SE. †$P < 0.05$, significant difference in tidal volume between low-altitude species.

Although there were small differences in absolute values between species, their patterns of acid-base regulation during both poikilocapnic and isocapnic hypoxia were the same: there did not appear to be any differences between species in the responses of pH, P_{CO_2}, or calculated bicarbonate concentrations of arterial blood (Fig. 5).

Ventilatory response to CO_2/pH

In addition to both poikilocapnic and isocapnic conditions, all species were exposed to hypercapnia (5% inspired CO_2) at 12% $F_{I_{O_2}}$. As a result, the CO_2/pH sensitivity of all three species could be assessed during moderate hypoxia by using

Table 3. *Respiratory variables during isocapnic hypoxia*

$F_{I_{O_2}}$	T_I	T_E	T_I/T_E	$P_{CL_{CO_2}}$
		Bar-headed goose		
21%O_2	2.35±0.16^a	3.48±0.25^a	0.69±0.06^a	36.7±4.4^a
12%O_2	2.09±0.11^b	2.54±0.18^b	0.83±0.05^{ab}	34.5±4.2^a
9%O_2	1.68±0.10^c	1.74±0.11^c	0.97±0.04^b	32.4±3.2^a
7%O_2	1.21±0.11^d	1.42±0.12^{cd}	0.86±0.02^{ab}	37.3±2.0^a
5%O_2	0.87±0.06^e	1.25±0.19^{cd}	0.73±0.05^a	37.1±1.1^a
4%O_2	0.92±0.08^e	1.13±0.05^d	0.82±0.07^{ab}	37.1±2.4^a
		Greylag goose		
21%O_2	2.32±0.16^a	3.04±0.22^a	0.79±0.07^a	42.0±1.9^a
12%O_2	1.93±0.21^a	2.05±0.24^b	0.96±0.05^b	39.7±1.5^{ab}
9%O_2	1.31±0.23^b	1.36±0.22^c	0.96±0.03^b	38.4±1.7^{ab}
7%O_2	1.04±0.09^b	1.02±0.09^c	1.02±0.05^b	36.1±1.2^c
5%O_2	1.02±0.12^b	0.96±0.09^c	1.06±0.08^b	34.4±1.1^c
		Pekin duck		
21%O_2	2.07±0.11^a	3.24±0.29^a	0.67±0.08^a	31.1±1.8^a
12%O_2	1.83±0.10^a	2.11±0.10^b	0.73±0.11^{ab}	28.7±1.9^b
9%O_2	1.29±0.04^b	1.50±0.07^c	0.87±0.03^{ab}	28.8±1.9^b
7%O_2	0.81±0.04^c	0.86±0.08^d	0.96±0.04^b	29.3±1.9^b
5%O_2	0.87±0.04^c	0.94±0.08^d	0.94±0.04^b	30.7±2.2^b

$F_{I_{O_2}}$, inspired O_2 fraction (%); T_I, inspiratory time (s); T_E, expiratory time (s); $P_{CL_{CO_2}}$, clavicular air sac CO_2 tension (Torr). Statistics were performed within each species only. $^{a,b,c,d,e}P < 0.05$, for each parameter, treatments with different letters are significantly different.

Table 4. *Blood gas variables during isocapnic hypoxia*

F_IO_2	Ca_{O_2}	Pv_{O_2}	Pv_{CO_2}	pHv	$[HCO_3^-]v$	Cv_{O_2}
			Bar-headed goose			
21%O_2	4.86±0.15[a]	38.9±2.8[a]	31.8±1.7[a]	7.37±0.05[a]	16.9±1.4[a]	2.19±0.28[a]
12%O_2	4.60±0.28[a]	42.6±5.7[a]	35.9±2.3[a]	7.36±0.06[a]	18.7±1.6[a]	1.99±0.25[a]
9%O_2	3.93±0.36[b]	37.9±1.3[a]	38.3±2.6[a]	7.24±0.10[ab]	16.0±3.1[a]	1.45±0.47[ab]
7%O_2	3.55±0.36[c]	32.4±2.5[a]	36.9±2.7[a]	7.21±0.07[ab]	13.4±1.2[ab]	1.95±0.18[a]
5%O_2	2.38±0.26[d]	17.6±1.7[b]	42.0±1.0[a]	6.99±0.09[b]	9.5±1.5[b]	0.47±0.17[b]
4%O_2	1.21±0.14[e]	14.2±2.1[b]	36.4±1.8[a]	6.93±0.10[b]	6.9±0.8[b]	0.12±0.06[b]
			Greylag goose			
21%O_2	5.62±0.26[a]	57.7±4.5[a]	31.5±1.6[a]	7.39±0.05[a]	17.7±2.7[a]	2.86±0.20[a]
12%O_2	5.13±0.18[a]	47.8±2.2[b]	29.4±1.5[a]	7.38±0.04[a]	15.8±0.7[ab]	2.76±0.16[a]
9%O_2	4.05±0.34[b]	40.2±2.5[b]	33.9±1.8[a]	7.24±0.03[ab]	13.2±0.4[ab]	1.80±0.38[b]
7%O_2	3.07±0.19[c]	24.2±3.8[c]	37.0±2.8[a]	7.12±0.02[b]	11.1±1.2[b]	0.63±0.16[c]
5%O_2	1.61±0.40[d]	19.6±3.7[c]	31.7±2.6[a]	7.01±0.01[b]	7.2±0.7[b]	0.13±0.08[c]
			Pekin duck			
21%O_2	5.04±0.18[a]	57.0±2.6[a]	33.9±1.1[a]	7.48±0.01[a]	22.8±1.4[a]	3.33±0.25[a]
12%O_2	4.17±0.15[b]	47.7±2.9[b]	36.8±2.0[ab]	7.36±0.07[a]	29.0±2.8[a]	2.75±0.43[ab]
9%O_2	3.82±0.13[b]	43.7±1.9[b]	38.3±1.2[ab]	7.40±0.05[a]	22.1±2.9[a]	2.23±0.21[b]
7%O_2	2.46±0.12[c]	34.1±3.6[c]	43.5±2.3[b]	7.25±0.03[ab]	17.0±2.5[ab]	1.35±0.12[c]
5%O_2	1.77±0.22[d]	21.8±3.3[d]	44.8±1.0[b]	7.06±0.08[b]	11.7±1.8[b]	0.31±0.11[d]

F_IO_2, inspired O_2 fraction (%); Ca_{O_2}, arterial O_2 content (mM); Pv_{O_2}, venous O_2 tension (Torr); Pv_{CO_2}, venous CO_2 tension (Torr); pHv, venous pH; $[HCO_3^-]v$, venous bicarbonate concentration (mM); Cv_{O_2}, venous O_2 content (mM). Statistics were performed within each species only. [a,b,c,d,e]$P < 0.05$, for each parameter, treatments with different letters are significantly different.

three different levels of Pa_{CO_2} and arterial pH (pHa). All three species increased ventilation via increases in both breathing frequency and tidal volume in response to increased Pa_{CO_2} and decreased pHa (Fig. 6). There were no statistically significant differences between species in the response of total ventilation to either Pa_{CO_2} or pHa (Fig. 6, *A* and *B*), but there was an insignificant trend of a reduced ventilatory sensitivity to reductions in pHa in greylag geese (due to a reduced tidal volume response). Curiously, Pa_{CO_2} was lower in greylag geese than in the other species during hypercapnia. Pekin ducks appeared to have a reduced breathing frequency response (Fig. 6*C*) and an enhanced tidal volume response (Fig. 6*E*) to Pa_{CO_2}, but these differences were not significant. Most importantly, there were no clear differences between bar-headed geese and the two low-altitude species during hypercapnic exposure.

Similar to the responses of Pa_{CO_2} and pHa to hypercapnia (Fig. 6), Pv_{CO_2} increased and venous pH (pHv) decreased compared with isocapnic levels (Table 5). Hypercapnia also had modest effects on blood oxygen levels. Pa_{O_2} was generally elevated by the enhancement of ventilation during hypercapnia compared with isocapnia, but this was not always accompanied by an increase in O_2 concentration (possibly due to effects of CO_2/pH on blood O_2 binding) (Table 5).

Breathing mechanics during hypoxia. A preliminary assessment of how breathing mechanics might influence the hypoxic ventilatory responses of each species was performed by analyzing the relationships between total ventilation and inspiratory/expiratory flow rates (using data from both poikilocapnia and isocapnia). There was an extremely strong correlation ($R^2 = 0.97$) between total ventilation and peak inspiratory flow using data from all three species (Fig. 7*A*). Notably, however, the maximum observed peak inspiratory flow in greylag geese was much lower than in the other species (dashed vertical line in Fig. 7*A*). The analogous correlation between total ventilation and peak expiratory flow was also strong ($R^2 = 0.96$), but the isocapnic data points for greylag geese at 7 and 5% F_{IO_2} deviated from the regression (Fig. 7*B*). Therefore, whereas the maximum observed peak expiratory flows in this species were 67–75% of those in bar-headed geese and pekin ducks (dashed vertical lines in Fig. 7), the maximum peak inspiratory flows were only 46–56%. Therefore, an inability to increase peak inspiratory flow may have limited the hypoxic ventilatory response of greylag geese, especially during severe isocapnic hypoxia. In particular, it may explain why greylag geese could not concurrently generate large tidal volumes and high breathing frequencies. These results were supported by flow-volume loops constructed from representative breath sequences of all individuals (data not shown), suggesting that limitations in the generation of inspiratory flow may exist across the breathing cycle in greylag geese.

Metabolic responses to hypoxia and elevated breathing. The rate of O_2 consumption increased in all species during both poikilocapnic and isocapnic hypoxia (Fig. 8), which likely reflects the metabolic costs of respiratory and cardiac muscle work. Furthermore, there was variability in the metabolic responses to hypoxia between species. During poikilocapnic hypoxia, bar-headed geese had the greatest increase in O_2 consumption and greylag geese had the lowest (Fig. 8*A*). Similar trends were observed during isocapnic hypoxia, but the differences between bar-headed geese and the other two species were less pronounced (Fig. 8*B*). As a result, only small differences between species were observed in their air convection requirements (total ventilation expressed relative to O_2 consumption rate, also called the ventilatory equivalent) during poikilocapnic hypoxia (Fig. 8*C*). This suggests that the species differences in total ventilation during severe hypoxia were due to differences in metabolic demands and that the higher total ventilation per se did not cause the enhanced O_2 loading in bar-headed geese during severe hypoxia (Fig. 1*B*). The enhanced tidal volume response (which suggests an increase in effective ventilation, see DISCUSSION) may instead account for the enhanced O_2 loading in this species (Fig. 2). Interestingly,

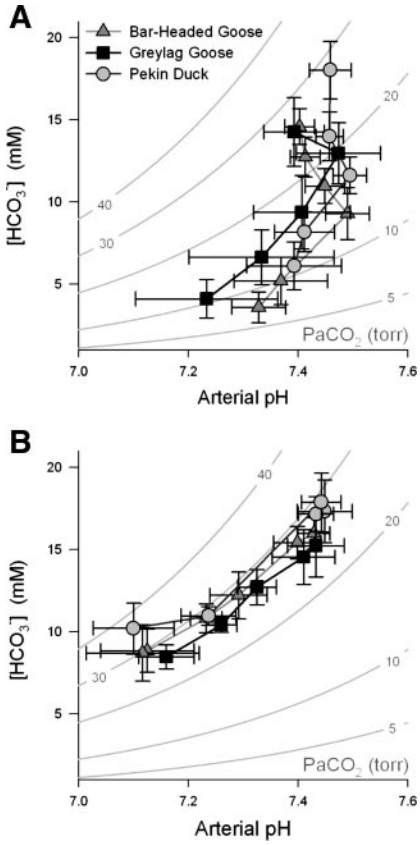

Fig. 5. Acid-base regulation was similar during both poikilocapnic (uncontrolled CO_2) and isocapnic (constant arterial CO_2) hypoxia in all species (bar-headed geese, greylag geese, pekin duck). *A*: during poikilocapnic hypoxia, no statistically significant pH imbalance occurred in arterial blood. *B*: during isocapnic hypoxia, all species experienced a metabolic acidosis of arterial blood at severe levels of hypoxia. Data are means ± SE.

air convection requirements were lower in bar-headed geese at 7% $F_{I_{O_2}}$ than in both other species (Fig. 8*D*).

DISCUSSION

During their biannual migration over the Himalayan mountains, bar-headed geese fly at altitudes up to 9,000 m, where oxygen levels in the air can be exceedingly low. Sustained flight elevates metabolic rate 10- to 20-fold above resting levels in birds and must be accompanied by similar increases in O_2 uptake. Our present findings suggest that among the respiratory adaptations that have evolved in bar-headed geese to facilitate O_2 loading during severe environmental hypoxia is an enhanced ventilatory response to poikilocapnic hypoxia (Fig. 1). Because this was primarily due to a larger tidal volume response (Fig. 2), there would have been a large enhancement of parabronchial (effective) ventilation. Pa_{O_2} and Ca_{O_2} were therefore substantially higher in bar-headed geese during severe hypoxia. This improved ability of bar-headed geese to load O_2 into the blood may at least partially account for their incredible ability to fly high.

We will consider several mechanisms that could account for the heightened breathing response to hypoxia in bar-headed geese. Chemoreceptors stimulate breathing in response to reductions in Pa_{O_2} (sensed by arterial chemoreceptors) as well as increases in Pa_{CO_2} and decreases in pH (sensed by arterial and central chemoreceptors) (7, 33, 43). During environmental hypoxia, Pa_{O_2} is reduced, stimulating increases in breathing. This augments CO_2 loss, however, which causes respiratory hypocapnia, partially offsetting the ventilatory response. The enhanced breathing response of bar-headed geese could therefore be due to an increased sensitivity to arterial hypoxia or a reduced sensitivity to hypocapnia (and any associated pH disturbance). Differences in ventilation between species also could have been caused by differences in the mechanical constraints on ventilatory flows or in the magnitude of the metabolic responses to hypoxia. All of these possibilities are discussed in more detail below.

Oxygen chemosensitivity is not enhanced in bar-headed geese. The enhanced ventilatory response of bar-headed geese to poikilocapnic hypoxia was probably not caused by an increased chemosensitivity to reductions in Pa_{O_2}. Ventilatory responses to isocapnic hypoxia are not inhibited by respiratory hypocapnia as they are during poikilocapnic hypoxia (because Pa_{CO_2} is held constant), making the isocapnic HVR a reasonable indicator of O_2 chemosensitivity. Because the isocapnic HVR was the same in bar-headed geese and pekin ducks (Fig. 3), we believe their chemoreceptor sensitivities to O_2 are similar. The isocapnic HVR in greylag geese was reduced compared with the other species, but this was probably due not to reduced O_2 chemosensitivity but to pulmonary mechanical constraints, as discussed below. This contrasts with reports in high altitude-adapted humans, where evolutionary alterations in O_2 chemosensitivity of breathing (both increases and decreases) have been demonstrated (8, 63) (see Introduction).

The isocapnic HVR in our experiment may not represent a sole effect of changes in arterial hypoxia per se for two reasons. First, because birds possess intrapulmonary CO_2 chemoreceptors (IPC), adding CO_2 to the inspired gas to maintain arterial isocapnia would have altered IPC discharge (32, 42). IPC discharge has slight inhibitory effects on breathing (but primarily alters breathing pattern), and discharge declines as CO_2 increases (14, 42). Although the net effect might be a slight stimulation of breathing, at elevated inspired CO_2, IPC discharge would be low in all species. We therefore anticipate that potential differences in IPC discharge had a minimal influence on our results in isocapnic hypoxia, bearing in mind that our understanding of the sensitivities and roles of IPCs in intact animals (particularly bar-headed geese) is restricted. Second, although Pa_{CO_2} was held constant during the isocapnic HVR experiment, all birds experienced a metabolic acidosis. This also could have stimulated breathing, so the measured isocapnic HVR may have been greater than a response to arterial hypoxia alone. Since the measured acidosis was similar in all birds, this effect was probably equivalent in all species.

Has sensitivity to CO_2 changed in bar-headed geese? The enhanced ventilatory response of bar-headed geese to poikilocapnic hypoxia was not caused by differences in Pa_{CO_2} or pHa, because changes in these variables during hypoxia were similar among species in both the current study (Fig. 5*A*) and those previously conducted (3). Both extracellular and intracellular acid-base regulation are exceptional in waterfowl (13, 14, 61), which could account for the tight regulation of pHa during respiratory hypocapnia. The responses of Pa_{CO_2} and pHa to isocapnic hypoxia were also similar between species (Fig. 5*B*). Unlike poikilocapnic hypoxia, however, all species exhibited a metabolic acidosis. The cause of this acidosis is unclear, but it

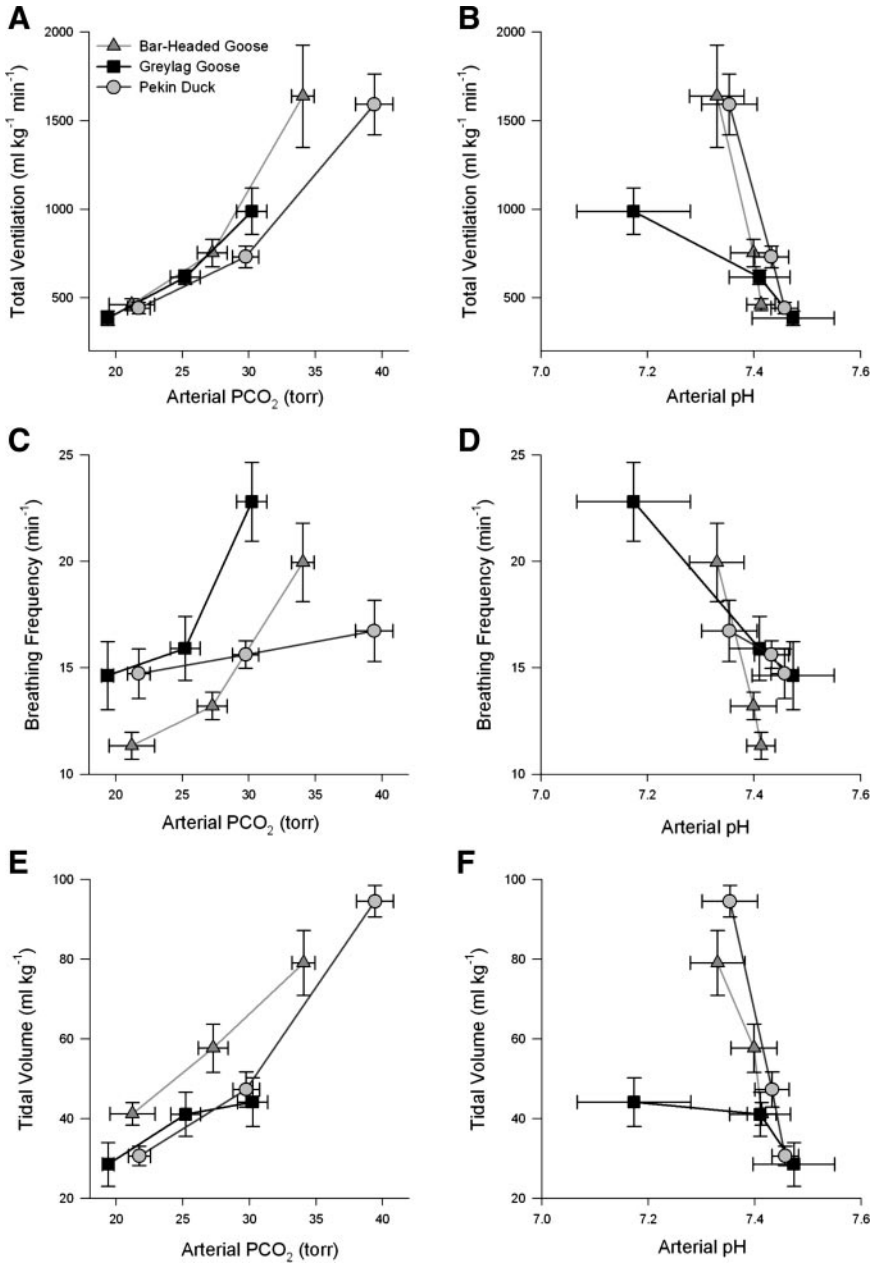

Fig. 6. Total ventilation (*A* and *B*), breathing frequency (*C* and *D*), and tidal volume (*E* and *F*) as a function of arterial CO_2 tension (P_{CO_2}; *A*, *C*, and *E*) or arterial pH (*B*, *D*, and *F*) in bar-headed geese, greylag geese, and pekin ducks. Data are means ± SE.

could have resulted from an increase in anaerobic metabolism or a hypoxemia-induced reduction in blood bicarbonate levels as occurs during poikilocapnic hypoxia.

There was no difference between species in the hypercapnic ventilatory response measured during moderate hypoxia. In all species at 12% $F_{I_{O_2}}$, breathing responded similarly to elevated Pa_{CO_2} and reduced pHa (Fig. 6). Curiously, the changes in breathing pattern in response to hypercapnia varied between species, but these differences were not statistically significant. Although these results suggest that reduced CO_2/pH chemosensitivity does not cause the enhanced poikilocapnic HVR in bar-headed geese, this suggestion is not unequivocal. There could still be reduced sensitivity to hypocapnia (reduced CO_2) but not hypercapnia (elevated CO_2). Furthermore, CO_2/pH sensitivity may also change as a function of Pa_{O_2} such that bar-headed geese may only be less sensitive to hypocapnia when severely hypoxic. Indeed, total ventilation in bar-headed geese appeared nearly independent of Pa_{CO_2} and pHa in severe hypoxia (compare Figs. 1 and 3). Interactions between O_2 and CO_2/pH sensitivity are well described in mammals (11), and it is likely that the nature of this interaction could change through evolution. However, such a low sensitivity to CO_2/pH is surprising and is not typical of other species of mammal or birds (7, 45); this may be suggestive of an interesting and adaptive control mechanism. A reduced chemosensitivity to hypocapnia may also explain the increased tidal volume and parabronchial ventilation, and therefore the enhanced O_2 loading, in bar-headed geese during severe hypoxia. We are unaware of any other examples of reduced hypocapnic chemosensitivity, but hypercapnic chemosensitivity is known to be lower in burrowing animals (which are chronically exposed to higher levels of CO_2) (38) and can increase (not decrease)

Table 5. *Blood gas variables during hypercapnia (5% inspired CO_2) at moderate hypoxia (12% inspired O_2)*

Pa_{O_2}	$[HCO_3^-]a$	Ca_{O_2}	Pv_{O_2}	Pv_{CO_2}	pHv	$[HCO_3^-]v$	Cv_{O_2}
			Bar-headed goose				
75.5±2.2	16.7±1.8	4.00±0.53	36.6±3.8	42.7±2.8	7.20±0.09	15.7±2.3	2.65±0.58
			Greylag goose				
68.1±1.8	10.5±1.8	5.12±0.23	55.4±5.7	37.1±2.0	7.12±0.11	11.8±3.0	2.62±0.37
			Pekin duck				
73.4±3.5	20.0±1.4	4.57±0.16	61.4±3.2	46.8±1.1	7.32±0.06	22.7±3.0	3.37±0.15

Pa_{O_2}, arterial O_2 tension (Torr); $[HCO_3^-]a$, arterial bicarbonate concentration (mM); Ca_{O_2}, arterial O_2 content (mM); Pv_{O_2}, venous O_2 tension (Torr); Pv_{CO_2}, venous CO_2 tension (Torr); pHv, venous pH; $[HCO_3^-]v$, venous bicarbonate concentration (mM); Cv_{O_2}, venous O_2 content (mM).

during ventilatory acclimatization to hypoxia in mammals (16, 21).

The effects of poikilocapnic hypoxia on ventilation have been studied previously in bar-headed geese and produced results that are largely consistent with our present findings: bar-headed geese increase ventilation more than pekin ducks in severe hypoxia (3, 5, 56). They also maintained higher rates of O_2 consumption (3), the importance of which is discussed below. In contrast to our present findings, however, previous studies found that pekin ducks started to increase ventilation at a higher FI_{O_2} levels than bar-headed geese. The reasons for this are not clear.

Pulmonary mechanics may have evolved in bar-headed geese. The ventilatory response of greylag geese appears to be limited by their capacity to generate high inspiratory flows (Fig. 7), possibly due to a reduced ability to generate high inspiratory pressure. This would reduce the capacity for greylag geese to increase tidal volume and breathing frequency concurrently and may account for this species' diminished tidal volume responses to hypoxia and hypercapnia (Figs. 2, 4, and 6). Human endurance athletes are known to approach the mechanical limits of their respiratory system for generating inspiratory pressure during maximal exercise (36). This and other mechanical limitations can reduce arterial O_2 loading, even in normoxia (12). Bar-headed geese attained higher peak inspiratory flows than both other species in the current study. During high-altitude flight, both hypoxia and exercise would intensely stimulate breathing, and the data suggest that bar-headed geese may have an enhanced mechanical capacity to generate high ventilatory flows, at least compared with their close relative, the greylag goose.

Hemoglobin has evolved in bar-headed geese. Bar-headed geese are known to have a higher inherent hemoglobin O_2 affinity than low-altitude waterfowl, primarily due to one amino acid point mutation in the α_A polypeptide chain (44, 58, 64). Bar-headed goose whole blood has a P_{50} value of 30 Torr at standard conditions in vitro, compared with 40 Torr in greylag geese (44). Bar-headed goose hemoglobin also lacks a salt bridge important for the Bohr effect (39). Interestingly, studies of bar-headed goose hemoglobin were some of the best early examples of adaptation at the molecular level (29). In vivo O_2 equilibrium curves from data in the present study illustrate these findings. The in vivo O_2 equilibrium curves for isocapnic hypoxia revealed a left-shifted P_{50} value for the bar-headed goose (38 Torr vs. 46 and 48 Torr in the other 2 species), whereas those for poikilocapnic hypoxia revealed similar P_{50} values for all three species (35, 33, and 36 Torr). Given the parallel changes in Pa_{CO_2} and pHa in all three species, this simply reflects the reduced Bohr effect in bar-headed geese. The potential adaptive benefit of a reduced Bohr effect in bar-headed geese is unclear. Although it may have arisen to restrict increases in O_2 affinity during hypocapnia/alkalosis (49), it also could reduce the beneficial effects of the Bohr effect on O_2 unloading to the tissues, which normally occurs during exercise-induced tissue acidosis.

The metabolic response to hypoxia has evolved in bar-headed geese. During hypoxia at rest, metabolism can change as a result of two processes: elevated O_2 demand by respiratory muscle (and possibly demand from cardiac muscle and tissue acid-base regulation) and reduced O_2 demand due to metabolic depression in other tissues (30). In the present study, the sum of these processes resulted in a net increase in metabolism during hypoxia in all three species (Fig. 8). Thus, although hypoxia depresses whole body metabolism in many other vertebrates, elevated metabolism occurs in some (but not all) bird species during hypoxia (3, 55), and this likely resulted from the augmented cardiorespiratory requirements for O_2 transport. Although no previous studies have directly assessed the cost of breathing during severe hypoxia in birds, some previous work has suggested that it might be appreciable. In Canada geese (*Branta canadensis*), tripling breathing frequency at constant tidal volume increases the metabolic costs of breathing approximately fourfold (26); tidal volume also increases substantially during hypoxia, which would further increase the cost of breathing (48). In elite human athletes at maximal exercise, both oxygen consumption and total ventilation increase 15- to 20-fold above resting levels (36). At these high rates of total ventilation, the metabolic costs of breathing can increase substantially and account for 15% or more of the total O_2 consumption rate (i.e., 15% of $\dot{V}O_{2\,max}$) (36). If that metabolic cost of breathing is compared with the total O_2 consumption rate at rest, just increasing ventilation would result in a 2.5- to 3-fold increase in O_2 consumption rate. These previous studies, as well as observations that blood flow to respiratory muscles increases severalfold during hypoxia in waterfowl (19), suggest that the increases in metabolic rate (i.e., O_2 consumption rate) observed in the present study (Fig. 8) can be partly attributed to the increased cost of breathing. Furthermore, the data also suggest that some of the difference between bar-headed geese and the other two species in the rise of metabolic rate observed at 5% FI_{O_2} may be attributed to differences in breathing.

Total ventilation generally increased in all species as the air they breathed became more hypoxic. Other than at 5% FI_{O_2}, total ventilation was similar between species, raising the question of what accounts for the higher metabolic rates in bar-

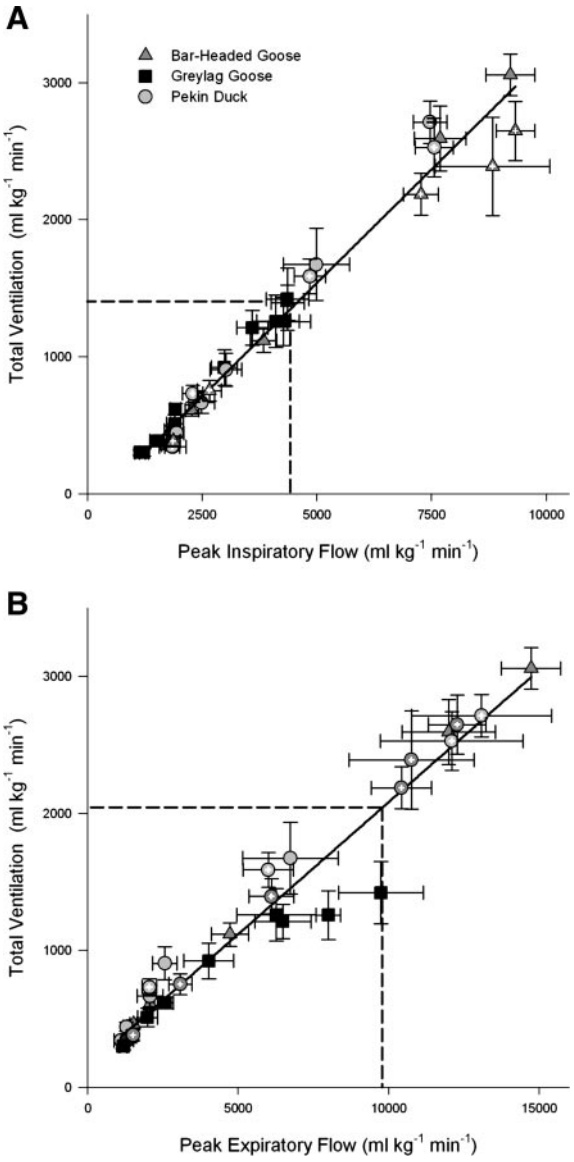

Fig. 7. Total ventilation relative to peak inspiratory flow (PIF; *A*) and peak expiratory flow (PEF; *B*) in bar-headed geese, greylag geese, and pekin ducks. *A*: there was a tight correlation ($R^2 = 0.97$) between ventilation and PIF in a regression including both poikilocapnic and isocapnic data from all species (symbols for isocapnic data are marked with +). *B*: there was also a tight correlation ($R^2 = 0.96$) between ventilation and PEF, but greylag geese fell off this regression line in severe isocapnic hypoxia (at 7 and 5% inspired F_{O_2}). Dashed vertical lines represent the maximum observed PIF and PEF values in greylag geese, and dashed horizontal lines represent the total ventilations predicted by the regression equations from these data.

headed geese at less severe levels of hypoxia. Since increasing ventilation via tidal volume is thought to be more costly than increasing ventilation via breathing frequency (41), the higher tidal volumes in bar-headed geese could partially explain their higher rates of metabolism. Breathing pattern aside, low-altitude waterfowl are known to restrict blood flow to the most hypoxia-sensitive tissues during hypoxia (i.e., heart and brain), but this does not occur in bar-headed geese (19, 20). Thus, despite net increases in total metabolism, low-altitude waterfowl may also have experienced some degree of regional metabolic depression, which may not have occurred in bar-headed geese. The ability of bar-headed geese to avoid metabolic depression in flight muscle during severe hypoxia is undoubtedly essential for high-altitude flight. Work in mammals suggests that hypoxic metabolic depression is controlled by hypothalamic sites that reduce metabolism, body temperature, and ventilation (1, 27). A relative metabolic depression in low-altitude species may therefore result from central inhibitory influences that also reduce the ventilatory response to hypoxia, which may not have occurred in bar-headed geese.

Effective ventilation is enhanced in bar-headed geese. The enhanced O_2 loading in bar-headed geese is best attributed to a large increase in parabronchial (effective) ventilation. With each breath, only part of the inspired air ventilates the gas exchange surface, while the rest only ventilates dead space. Total ventilation is thus composed of both effective ventilation and dead space ventilation. Deeper breaths (i.e., larger tidal volume breaths) reduce the contribution of total ventilation that ventilates dead space and will therefore result in greater effective ventilation of the gas exchange surface (assuming there are no large differences in anatomical dead space volume between species). Total ventilation and metabolism were evenly matched in all species during poikilocapnic hypoxia, because air convection requirements (total ventilation normalized to O_2 consumption rate) were similar (Fig. 8). This suggests that differences in total ventilation per se did not enhance arterial P_{O_2} or O_2 loading in bar-headed geese; total ventilation merely kept pace with the higher metabolic rate sustained by this species in hypoxia. The difference in parabronchial (effective) ventilation between bar-headed geese and low-altitude waterfowl was probably even greater than the difference in total ventilation, however, because bar-headed geese had higher tidal volumes and lower breathing frequencies at any given total ventilation during hypoxia (Fig. 2). This should increase the ratio of parabronchial ventilation to metabolism in bar-headed geese compared with low-altitude waterfowl and therefore cause the enhanced arterial P_{O_2} and O_2 loading during severe hypoxia.

Implications for high-altitude flight. During steady flight in normoxia, bar-headed geese and other birds increase their rate of O_2 consumption between 10- and 20-fold (2, 9, 57). This appears to be matched by a nearly equivalent increase in ventilation (2) such that air convection requirements are approximately constant between rest and flight exercise (at least in steady state). It is unknown whether this is also true of flight in hypoxia, since no previous studies have measured breathing or metabolism under these combined conditions. However, it raises the question of whether these animals can increase ventilation in hypoxia 10-fold to meet resting metabolic demands and then another 10- to 20-fold to accommodate the metabolic costs of flight. In other words, can the hypoxic ventilatory response be fully sustained during the exercise of flight? The air convection requirement increases in a similar fashion going from rest to exercise during running in bar-headed geese and pekin ducks in both normoxia and hypoxia (23, 37), suggesting that hypoxia and exercise normally have additive effects on ventilation. Bar-headed geese may therefore be capable of exceptionally high maximum ventilation rates, but the upper limits of this species' ventilatory system have yet to be determined.

Another set of intriguing questions arises from the fact that breathing frequency is coordinated with wing beat frequency

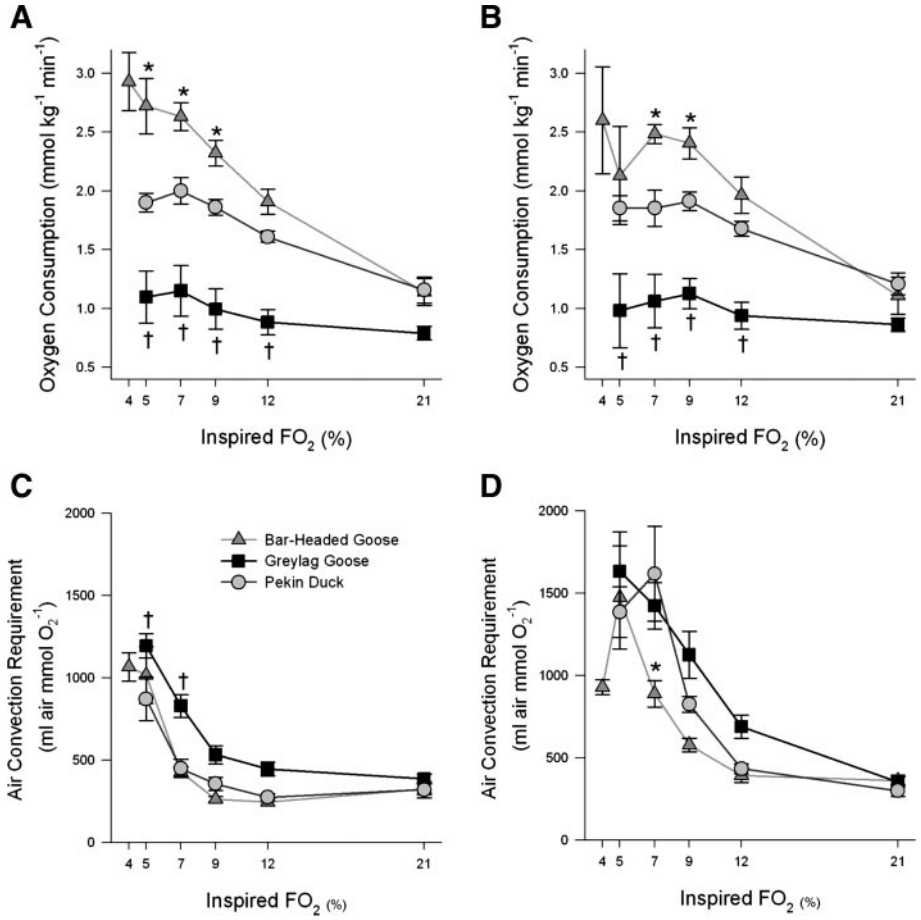

Fig. 8. Responses of O_2 consumption rate to poikilocapnic (uncontrolled CO_2) hypoxia (*A*) and isocapnic (constant arterial CO_2) hypoxia (*B*) were different between species. *C*: there were small differences between greylag geese and the other 2 species (bar-headed geese and pekin ducks) during poikilocapnic hypoxia when total ventilation was expressed relative to O_2 consumption rate (air convection requirement, also called the ventilatory equivalent). *D*: air convection requirement was lower in bar-headed geese at 7% inspired F_{O_2}. Data are means ± SE. *$P < 0.05$, significant difference between bar-headed geese and both low-altitude species. †$P < 0.05$, significant difference between low-altitude species.

during flight (6). This coordination is thought to reduce the energetic costs of breathing (26), and geese are known to exhibit wing beat/respiration ratios between 1:1 and 4:1 (10, 24, 25). This raises the reciprocal questions of whether respiratory-locomotor coupling is disrupted by hypoxia or whether the hypoxic ventilatory response is disrupted by respiratory-locomotor coupling. Within this context is the question of how the differences we observed between the breathing pattern responses of bar-headed geese and the other species at rest apply during flight. The data do suggest that altering respiratory drive will alter the entrainment ratio (from 1:1 to 2:1 for instance) (24), suggesting that flying birds can adjust their breathing patterns to enhance O_2 loading into the blood and still coordinate breathing with wing flapping.

Finally, the partial pressure of O_2 is reduced at high altitude because of declines in total barometric pressure (hypobaria) rather than reduced $F_{I_{O_2}}$. An important, and as yet unanswered, question is how hypobaria influences breathing and O_2 loading in bar-headed geese. Previous studies comparing the responses to normobaric and hypobaric hypoxia suggest that parabronchial ventilation, aerodynamic valving, and O_2 loading are largely unaffected by reductions in barometric pressure alone (50, 51). Analogous experiments have not been conducted on bar-headed geese, and they have not been conducted during flight in any species. It is therefore possible that pulmonary mechanics and O_2 loading may be affected by hypobaria at rest or during flight in this species, and this certainly deserves future study.

Can neutral phylogenetic variation explain the interspecific differences? Bar-headed geese, greylag geese, and ross geese (*Anser rossii*) form a monophyletic group within the genus *Anser* (subfamily Anserinae), so greylag geese are a close evolutionary relative of bar-headed geese (15). Pekin ducks are more distantly related and belong to a neighboring subfamily (Anatinae). There are undoubtedly many characteristics that have diverged between geese and ducks that can be explained by neutral evolutionary processes such that greylag geese and bar-headed geese are more alike to one another than to pekin ducks (28). With regard to the potential limitation for generating inspiratory airflow in greylag geese, this observation was probably not caused by neutral evolution but is inconsistent with hypoxia adaptation. However, pekin ducks and greylag geese were similar in the majority of their responses to hypoxia, whereas bar-headed geese were often different. This suggests that the enhanced effective ventilation and O_2 loading of bar-headed geese to hypoxia were not caused by neutral evolution and that these unique phenotypes may be related to the exceptional hypoxia tolerance of this species.

The Himalayan mountains are a formidable barrier to avian migration, and many species that migrate between the northern and southern sides of the range fly around or employ longer routes through riverine valleys (35, 52, 53). Bar-headed geese routinely migrate along direct routes over the highest peaks in the Himalayas, an exceptional feat as exemplified by its rarity amongst migrating Asian birds. The extreme nature of this migration, along with the known importance of breathing for

O_2 transport in hypoxia, strongly suggests that the enhanced effective ventilation and O_2 loading of this species are adaptive.

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