The Company of Biologists

RESEARCH ARTICLE

Divergent respiratory and cardiovascular responses to hypoxia in bar-headed geese and Andean birds

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ABSTRACT

Many high-altitude vertebrates have evolved increased capacities in their oxygen transport cascade (ventilation, pulmonary diffusion, circulation and tissue diffusion), enhancing oxygen transfer from the atmosphere to mitochondria. However, the extent of interspecies variation in the control processes that dictate hypoxia responses remains largely unknown. We compared the metabolic, cardiovascular and respiratory responses to progressive decreases in inspired oxygen levels of bar-headed geese (*Anser indicus*), birds that biannually migrate across the Himalayan mountains, with those of Andean geese (*Chloephaga melanoptera*) and crested ducks (*Lophonetta specularioides*), lifelong residents of the high Andes. We show that Andean geese and crested ducks have evolved fundamentally different mechanisms for maintaining oxygen supply during low oxygen (hypoxia) from those of bar-headed geese. Bar-headed geese respond to hypoxia with robust increases in ventilation and heart rate, whereas Andean species increase lung oxygen extraction and cardiac stroke volume. We propose that transient high-altitude performance has favoured the evolution of robust convective oxygen transport recruitment in hypoxia, whereas life-long high-altitude residency has favoured the evolution of structural enhancements to the lungs and heart that increase lung diffusion and stroke volume.

KEY WORDS: Altitude, Ventilation, Cardiac output, Metabolism, Goose, Duck

INTRODUCTION

Hypoxia (low environmental oxygen) at high altitude is challenging to vertebrate life, and is believed to have driven adaptations that improve oxygen transport in high-altitude species. Two high-altitude populations that have received decades of scientific scrutiny are Tibetan and Andean humans, who exhibit strikingly divergent strategies for coping with hypoxia, which include changes in control processes that dictate hypoxia responses. Tibetans have high resting levels of ventilation and a robust ventilatory response to hypoxia compared with humans from lowland populations (Beall, 2002), but a normal haemoglobin concentration ([Hb]) and haematocrit (Hct) (Beall, 2007). Conversely, Andeans have a substantially lower hypoxic ventilatory response than Tibetans and lowlanders (Beall et al., 1997; Beall, 2007), and instead exhibit an enhanced blood-oxygen carrying capacity (higher [Hb] and higher arterial oxygen content, $C_{a_{O_2}}$) (Beall, 2007) and greater lung volume and pulmonary diffusion capacity compared with lowlanders (Brutsaert et al., 2000). Notably, Tibetans tend to suffer much less than Andeans from high-altitude diseases such as chronic mountain sickness, which is believed to result from off-target responses to chronic hypoxia (Monge et al., 1992; Simonson, 2015), suggesting that differences in the control processes that dictate hypoxia responses likely have important implications for fitness at high altitude. The extent to which other high-altitude populations employ convergent versus divergent strategies for dealing with high altitude is poorly understood.

Birds have evolved cardiorespiratory specializations permitting greater hypoxia tolerance in general than mammals. These features are distributed throughout the oxygen transport cascade, the steps conceptualizing oxygen transfer from the atmosphere to the mitochondria (ventilation, lung oxygen diffusion, circulation and tissue oxygen diffusion). Bird lungs have a larger surface area and thinner diffusion barrier than mammalian lungs and a unique gas exchange system that is more efficient than the mammalian system (Shams and Scheid, 1989; Scheid, 1990; Scott, 2011). Birds tolerate hypocapnia (reduced blood CO_2) better than mammals (Scheid, 1990), permitting greater ventilation increases during hypoxia. Their larger hearts can produce greater cardiac output than those of mammals (Calder, 1968; Grubb, 1983; Smith et al., 2000), and they have increased tissue capillary exchange capacity (Scott et al., 2015).

Bar-headed geese (*Anser indicus*) migrate across the Himalayas (routinely at altitudes from 4500 to 5500 m, and occasionally at altitudes above 7200 m) between breeding grounds on the Mongolian and Tibetan plateaus (2000–4000 m) and low-altitude overwintering sites in India (<1000 m) (Hawkes et al., 2011; Bishop et al., 2015). This species has evolved additional oxygen transport enhancements: larger lungs (the parabronchial lung not including the air sacs; Scott, 2011), more pronounced ventilation increases during hypoxia (Black and Tenney, 1980a; Scott and Milsom, 2007; Lague et al., 2016), Hb with an increased affinity for binding oxygen (Scott et al., 2009; McCracken et al., 2010), increased flight and cardiac muscle capillarity (Fjeldså and Krabbe, 1990; Scott, 2011), and mitochondrial redistribution reducing oxygen diffusion distances (Fjeldså and Krabbe, 1990).

Many other birds spend their whole lives at high altitude and it is unknown whether they have evolved similar mechanisms for enhancing oxygen transport. Andean geese (*Chloephaga melanoptera*) and crested ducks (*Lophonetta specularioides*), two

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Journal of Experimental Biology

List of symbols and abbreviations

Ca_{O_2}	oxygen content of arterial blood
Cv_{O_2}	oxygen content of venous blood
$F_{EC_{O_2}}$	fractional oxygen content of expired gas from the chamber
$F_{E_{O_2}}$	fractional oxygen content of expired gas of the bird
$F_{I_{O_2}}$	fractional inspired oxygen content of the bird
[Hb]	haemoglobin concentration
$[HCO_3^-]_a$	arterial bicarbonate ion concentration
Hct	haematocrit
Pa_{O_2}	arterial partial pressure of oxygen
pH_a	arterial pH
$P_{I_{O_2}}$	partial pressure of inspired oxygen
RQ	respiratory quotient (the ratio of CO_2 produced to O_2 consumed)
$\dot{V}_C$	rate of gas flowing through the chamber
$\dot{V}_{O_2}$	volume of oxygen consumed by the animal
$\dot{V}_R$	total ventilation

relatively unrelated taxa, are lifelong high-altitude residents of the Andes (3500–5500 m). They routinely fly, but do not migrate vast distances (Scott et al., 2009; Natarajan et al., 2015). Andean geese have evolved a Hb with an even greater affinity for oxygen than that of bar-headed geese (Black and Tenney, 1980a; Scott et al., 2009; McCracken et al., 2010; Lague et al., 2016), but little else is known about their physiology. We designed this study to determine whether Andean geese and crested ducks meet the challenge of high-altitude life using strategies convergent or divergent with those of bar-headed geese. To examine this, we compared how hypoxia controls the metabolic, ventilatory and cardiovascular systems between bar-headed geese, Andean geese and crested ducks. All measurements in this study were made at a common altitude (3200 m) in the field, where the only variation in ambient conditions was modest daily fluctuations in barometric pressure (which was corrected for) and temperature (which remained within the thermoneutral zones of the birds).

MATERIALS AND METHODS
Animals
Measurements were made on seven wild bar-headed geese, *Anser indicus* (Latham 1790) (2.1±0.1 kg), captured at Lake Qinghai, China (~3200 m), and on seven wild Andean geese, *Chloephaga melanoptera* (Eyton 1838) (2.1±0.1 kg), and six wild crested ducks, *Lophonetta specularioides* (King 1828) (0.98±0.04 kg), captured in the Andes (>4000 m). Bar-headed geese were studied 1–2 days after capture, and the Andean birds were studied after acclimating to ~3200 m for 6 months in San Pedro de Casta, Peru. All animals were housed in outdoor pens under natural conditions. Experiments were conducted at times of year outside the annual periods of migration and breeding. The bar-headed geese were at the end of their annual wing moult and the Andean geese were post-moult. All birds were adults of mixed sex (male/female: bar-headed geese, 4/3; Andean geese, 2/5; crested ducks, 5/1). All experiments were conducted at 3200 m in the field at the respective sites. All procedures were conducted according to guidelines approved by the Animal Care Committee at the University of British Columbia (protocols A12-0013 and A16-0019) in accordance with the Canadian Council on Animal Care.

Surgical procedures
Surgery was conducted under general and local anaesthesia 1 day before the hypoxic exposure. All birds were first weighed, gently restrained and induced with isoflurane (4%) supplemented with O_2 (100%) by facemask prior to intubation. General anaesthesia was maintained with isoflurane and O_2. The right brachial artery and vein

were accessed via a small incision and blunt dissection and cannulated with polyurethane cannulae (PU-90; 0.102 cm internal diameter× 0.410 cm outer diameter) filled with 1000 IU ml^{-1} heparinized saline. The venous catheter was advanced to sit just outside the heart to sample mixed venous blood. Birds were recovered for at least 24 h, during which food was not provided to ensure birds were post-absorptive during experiments to help standardize the results.

Experimental protocol
Each bird was placed in a flexible cradle with its head in a 2 l flow-through respirometry chamber supported by the cradle and sealed around the neck with a latex collar (Lague et al., 2016). Birds were lightly restrained and were allowed 60–90 min to adjust to their surroundings under ambient conditions. All experiments were run in unheated facilities at ambient temperature (roughly 18–24°C, well within the thermoneutral zones of these birds) with a partial pressure of inspired oxygen ($P_{I_{O_2}}$) of ~14.7 kPa (barometric pressure at the sites was roughly ~500 mmHg, 70 kPa). Birds were then exposed to 25 min step reductions in $P_{I_{O_2}}$ achieved by mixing N_2 and air through calibrated rotameters. Multiplying the percentage O_2 in the resulting mix by the barometric pressure yielded $P_{I_{O_2}}$ values of 12.2, 9.1 and 7.1 kPa, and 5.1 kPa for Andean geese and bar-headed geese only (simulating elevations of approximately 4500, 7000, 8500 and 11,000 m) (Bouverot, 1985), and then recovered for 25 min at ambient $P_{I_{O_2}}$. All delivered gas was dry, and was delivered at a flow rate of 5–10 l min^{-1}. We chose to simulate a progressively increasing severity of hypoxia to permit better comparison with related studies in the literature that all use similar progressive hypoxic protocols (Black and Tenney, 1980a; Scott and Milsom, 2007; Lague et al., 2016).

Measurements
Rates of oxygen consumption indicative of metabolic rate were measured using flow-through respirometry and ventilation was measured using pneumotachography. These are traditional and well-established techniques (Black and Tenney, 1980a,b; Scott and Milsom, 2007; Hawkes et al., 2014; Lague et al., 2016). Whole-animal oxygen consumption rate ($\dot{V}_{O_2}$) was calculated from the difference between the fractional oxygen composition of inspired gas ($F_{I_{O_2}}$) and the fractional oxygen composition of expired gas from the chamber ($F_{EC_{O_2}}$), which were directly measured by a gas analyser (Sable Systems, Las Vegas, NV, USA), multiplied by the rate of gas flowing through the chamber ($\dot{V}_C$). Tidal volume and breathing frequency were measured from the head chamber outflow using a pneumotachograph connected to a differential pressure transducer (Validyne, Northridge, CA, USA) (Fleisch, 1925). Mean arterial pressure was continuously monitored using a pressure transducer (Deltran, Utah Medical Products Inc., Midvale, UT, USA) connected to the arterial cannula. Sampling of arterial and venous blood (0.4 ml each) occurred 15 min after exposure to each $P_{I_{O_2}}$, and after 5 and 25 min of recovery. Blood samples were immediately analysed and any remaining blood was returned to the bird. Arterial oxygen content (Ca_{O_2}) and venous oxygen content (Cv_{O_2}; mmol l^{-1}) were measured in triplicate at 41°C using the Tucker method (Tucker, 1967) using a FireSting oxygen probe (PyroScience, Aachen, Germany) (Lague et al., 2016). [Hb] (g dl^{-1}), Hct (%), arterial partial pressure of oxygen (Pa_{O_2}; kPa), arterial pH (pH_a) and arterial bicarbonate ion concentration ([HCO_3^-]$_a$; mmol l^{-1}) were analysed from arterial blood at 41°C using CG8+ cartridges with the i-STAT VetScan Analyzer (Abaxis, Union City, CA, USA). All i-STAT values were corrected according to Harter et al. (2015). We used the correction factors determined for bar-headed geese for all species, assuming that these corrections

should apply for all nucleated bird blood. $[HCO_3^-]_a$ was calculated using the Henderson–Hasselbalch equation, assuming a pK of 6.090 and a CO_2 solubility coefficient of 0.2117 mmol l^{-1} kPa^{-1} in plasma (Helbecka et al., 1964).

Data and statistical analysis

All data (except blood variables) were acquired and analysed using PowerLab data acquisition and analysis software (ADInstruments, Colorado Springs, CO, USA) at a sampling frequency of 1000 s^{-1}. Mean values were calculated 1–2 min prior to blood sampling.

The following variables were either directly measured or calculated. Tidal volume (ml kg^{-1}) and breathing frequency (min^{-1}) were derived from the integrated differential pressure signal and total ventilation ($\dot{V}_R$; ml min^{-1} kg^{-1}) was calculated from their product. $\dot{V}_{O_2}$ (ml min^{-1} kg^{-1}) was calculated as $\dot{V}_C \times (F_{I_{O_2}} - F_{E_{CO_2}})$ and standardized by body mass (kg). Water vapour was removed prior to gas analysis (Withers, 1977), and the respiratory quotient (RQ) was assumed to be 1.0.

Air convection requirement was the quotient of $\dot{V}_R$ and $\dot{V}_{O_2}$; i.e. the amount of air respired for each ml of O_2 consumed. Lung oxygen extraction (%; the percentage of O_2 contained in each breath that was extracted at the lung before the air was expired) was calculated as $[(F_{I_{O_2}} - F_{E_{O_2}})/F_{I_{O_2}}] \times 100$, where the fractional expired level of oxygen of the bird ($F_{E_{O_2}}$) was calculated as:

$$F_{E_{O_2}} = \frac{(\dot{V}_R \times F_{I_{O_2}}) - \dot{V}_{O_2}}{\dot{V}_R} .$$

Volume measurements were corrected for changes in barometric pressure and air density (Dejours, 1975). $\dot{V}_{O_2}$, air convection requirement and lung oxygen extraction were reported at standard temperature and pressure, dry (STPD). Total ventilation and tidal volume were reported at body temperature and pressure, saturated (BTPS), assuming a constant body temperature of 41°C.

Heart rate (min^{-1}) was calculated from the pulsatile arterial pressure trace. Mean arterial pressure (kPa) was the sum of diastolic pressure plus 1/3 the pulse pressure (systolic pressure minus diastolic pressure). Cardiac output (ml min^{-1} kg^{-1}) was calculated from the Fick equation using $\dot{V}_{O_2}$ and $Ca_{O_2} - C\bar{v}_{O_2}$. Stroke volume (ml kg^{-1}) was the quotient of cardiac output and heart rate. Total peripheral resistance (kPa min kg ml^{-1}) was the quotient of mean arterial pressure and cardiac output (Bech and Nomoto, 1982). Blood convection requirement (ml of blood pumped by the heart for each ml of O_2 consumed) was the quotient of cardiac output and $\dot{V}_{O_2}$. Tissue oxygen extraction (%) was calculated as $[(Ca_{O_2} - C\bar{v}_{O_2})/Ca_{O_2}] \times 100$.

Within each species, all data were analysed using one-way repeated measures analysis of variance (ANOVA) and Holm–Sidak *post hoc* tests. Comparisons between species were made using two-way (species and $P_{I_{O_2}}$) repeated measures ANOVA and Holm–Sidak *post hoc* tests within each $P_{I_{O_2}}$. $P<0.05$ determined statistical significance. Repeated measures ANOVA were used to provide a conservative indication of significance between exposures and between groups, increasing statistical power and reducing the risk of a type II error. Variables analysed with a one-way repeated measures ANOVA that failed normality or equal variance assumptions were transformed to meet these assumptions with $x'=\ln(x)$ for Andean geese ($\dot{V}_{O_2}$, $\dot{V}_R$, blood convection requirement, tissue oxygen extraction) and bar-headed geese ($\dot{V}_R$, cardiac output, $[HCO_3^-]_a$). $x'=x^2$ transformed $[HCO_3^-]_a$ for crested ducks and $x'=-1/x^2$ transformed mean arterial pressure in Andean geese. Similarly,
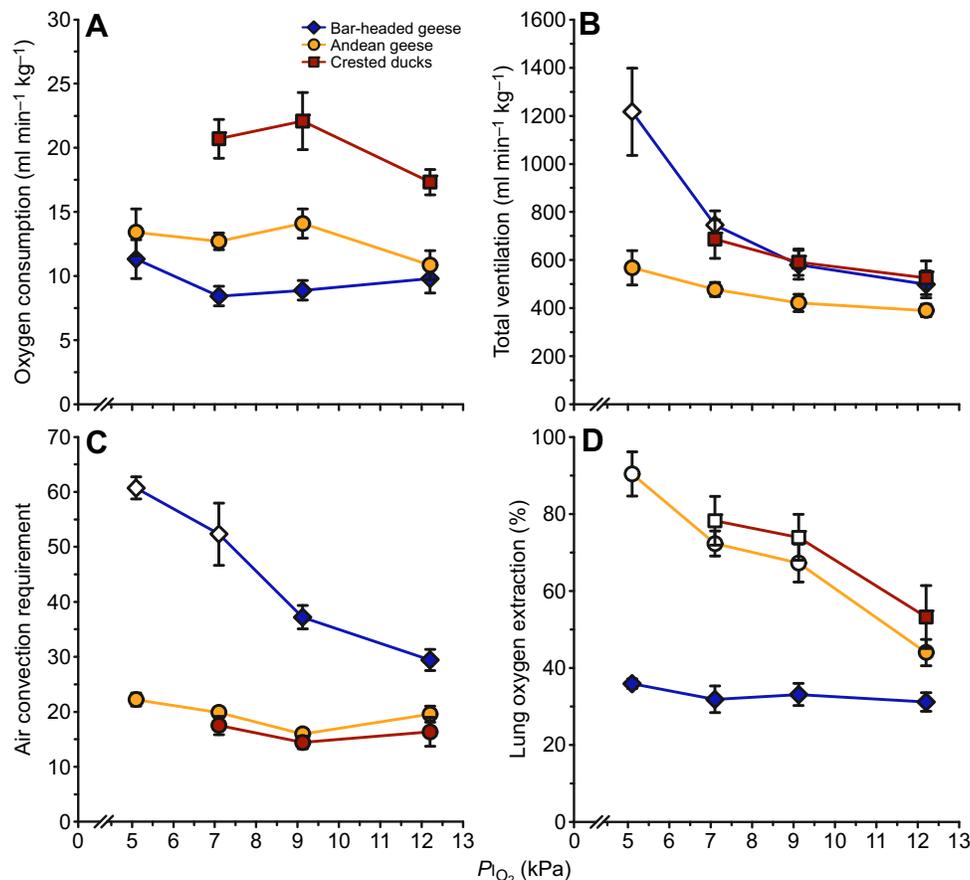

Fig. 1. Oxygen consumption, total ventilation, air convection requirement and lung oxygen extraction during progressive hypoxia. The relationship between inspired partial pressure of oxygen ($P_{I_{O_2}}$) and (A) oxygen consumption rate (standard temperature and pressure, dry: STPD), (B) total ventilation, (C) air convection requirement (the ratio of ml of air breathed to extract 1 ml of O_2), and (D) lung oxygen extraction (B–D, in body temperature and pressure, saturated: BTPS). All values are means±s.e.m. (bar-headed geese, N=7; Andean geese, N=7; crested ducks, N=6). Significant differences ($P<0.05$) from starting values within a species are indicated by open symbols.
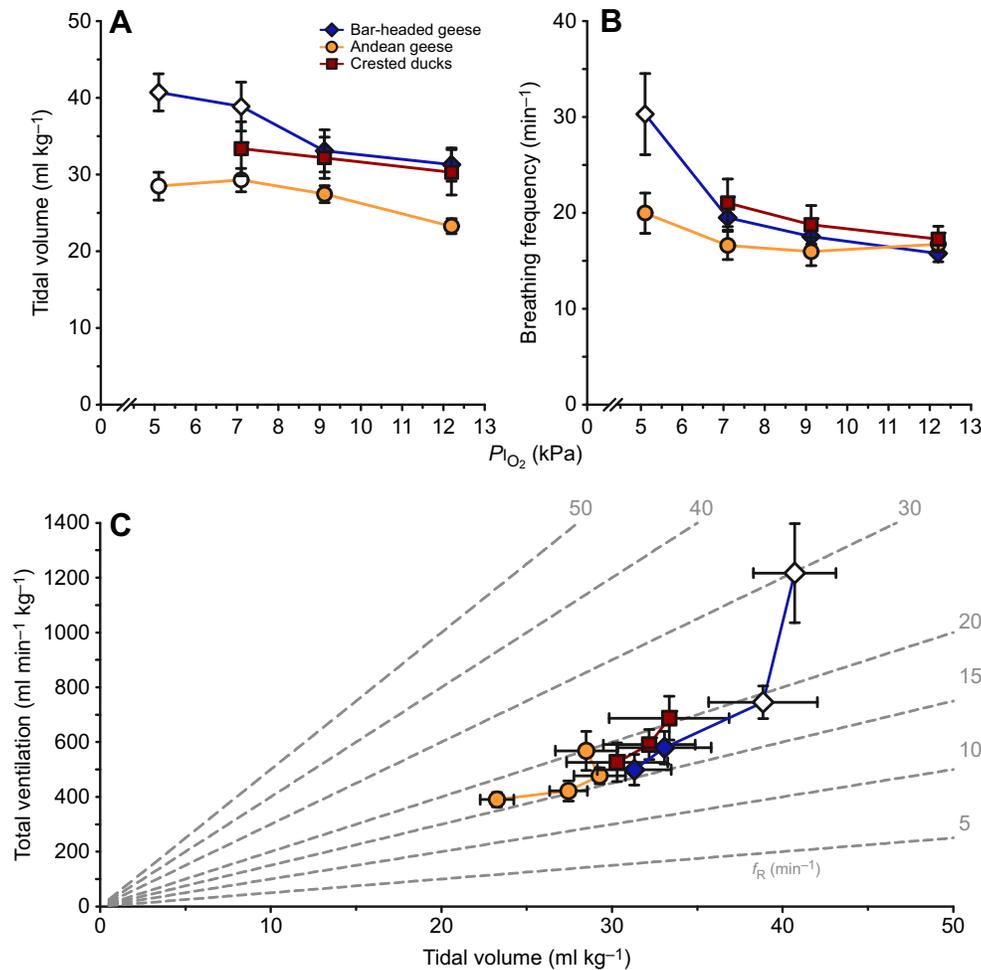
Fig. 2. Hypoxic ventilatory response patterns. (A,B) The relationship between inspired partial pressure of oxygen ($P_{I_{O_2}}$) and (A) tidal volume (in BTPS) and (B) breathing frequency. (C) Changes in total ventilation (in BTPS) and its components, tidal volume (x-axis) and breathing frequency (f_R; dashed isopleths), during exposure to progressive hypoxia. All values represent means±s.e.m. (bar-headed geese, N=7; Andean geese, N=7; crested ducks, N=6). Significant differences (P<0.05) in total ventilation from starting values within a species are indicated by open symbols.

two-way repeated measures ANOVA transformations to meet assumptions of normality and equal variance included $x'=\ln(x)$ ($C_{a_{O_2}}$, cardiac output, heart rate, stroke volume), $x'=\sqrt{x}$ ($C_{a_{O_2}}$) and a Box–Cox transformation with λ=−1.5 (pH$_a$). All other variables originally met normality and equal variance assumptions. Student t-tests compared Hct and [Hb] prior to and following each experiment. Statistical analyses were carried out using SigmaStat (version 3.0; Systat Software).

RESULTS

Metabolic and ventilatory responses

Bar-headed geese, Andean geese and crested ducks maintained their initial rates of oxygen consumption throughout progressive hypoxia ($P_{I_{O_2}}$=12.2, 9.1, 7.1 and 5.1 kPa), even during extreme hypoxic exposures that simulated $P_{I_{O_2}}$ levels equivalent to or higher than the top of Mount Everest ($P_{I_{O_2}}$ roughly 6.9 kPa; Fig. 1A). Remarkably, neither Andean geese nor crested ducks increased total ventilation significantly despite the severity of hypoxia (Fig. 1B). As a result, the air convection requirement, the ratio of ventilation to the rate of oxygen consumption, remained unchanged in Andean geese (P=0.098) and crested ducks (P=0.703) (Fig. 1C). In contrast, bar-headed geese maintained their routine oxygen consumption rate through >2-fold increases in the air convection requirement (P<0.001; Fig. 1C), in association with ~2.5-fold increases in total ventilation (P<0.001; Fig. 1B), that were mediated by increases in both tidal volume (P<0.001) and breathing frequency (P<0.001; Fig. 2).

With each decrease in $P_{I_{O_2}}$, lung oxygen extraction (the amount of oxygen extracted by the animal from the inspired air) progressively increased in Andean geese (P<0.001) and crested ducks (P=0.024), achieving maximum levels approaching 90% (Fig. 1D). Conversely, lung oxygen extraction was unchanged in bar-headed geese (P=0.241) and remained at a much lower level (~40%) (Fig. 1D). Lung oxygen extraction was similar in Andean geese and crested ducks (P=0.748), and significantly greater than that of bar-headed geese (P<0.001). Thus, the non-migratory, high-

Table 1. Comparison of arterial partial pressure of oxygen ($P_{a_{O_2}}$) values measured and corrected using Harter et al. (2015)

	$P_{I_{O_2}}$ (kPa)	$P_{a_{O_2}}$ (kPa)	
		Uncorrected	Corrected
Bar-headed geese	12.2	6.6±0.2	9.5±0.3
	9.1	4.8±0.1	6.9±0.2
	7.1	3.8±0.1	5.6±0.2
	5.1	3.2±0.2	4.7±0.3
Andean geese	12.2	6.7±0.2	9.6±0.3
	9.1	4.4±0.3	6.4±0.4
	7.1	3.4±0.2	5.0±0.2
	5.1	2.5±0.1	4.3±0.1
Crested ducks	12.2	5.5±0.5	7.9±0.6
	9.1	4.2±0.4	5.8±0.3
	7.1	3.8±0.7	5.1±0.5

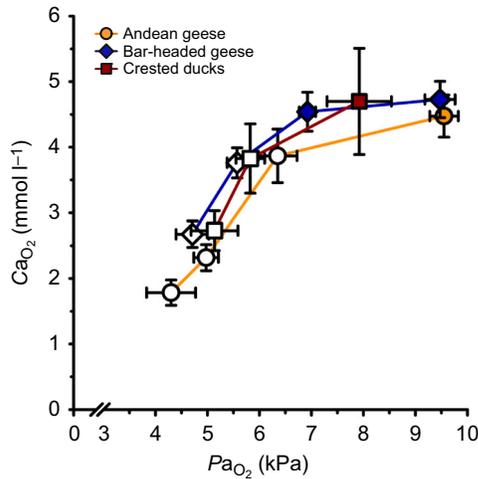All values represent means±s.e.m. (bar-headed geese, N=7; Andean geese, N=7; crested ducks, N=6).

Fig. 3. Arterial oxygen content (Ca_{O_2}) during progressive hypoxia. Ca_{O_2} decreased with decreasing arterial partial pressure of oxygen (Pa_{O_2}), producing *in vivo* oxygen equilibrium curves. All values represent means± s.e.m. (bar-headed geese, N=7; Andean geese, N=7; crested ducks, N=6). Significant differences (P<0.05) from starting values within a species are indicated by open symbols.

altitude resident Andean species (Andean geese and crested ducks) have evolved markedly different strategies for maintaining oxygen supply during severe hypoxia compared with the transiently high-altitude, long-distance migrating Himalayan bar-headed geese (increased lung oxygen extraction versus ventilation).

Blood gases and acid–base status

The corrected values of Pa_{O_2} decreased in all species as PI_{O_2} decreased (Table 1), and the *in vivo* blood-oxygen equilibrium curves were similar among the three species (Fig. 3), indicating that the two different ventilatory strategies for maintaining oxygen uptake were equally effective at blood-oxygen loading. Both Ca_{O_2} and Cv_{O_2} decreased significantly during hypoxia exposure in bar-headed geese (P<0.001), Andean geese (P<0.001) and crested ducks (P=0.016; Table 2). The arterial pH (pH_a) at each hypoxic step was significantly more alkalotic in bar-headed geese than in Andean geese (P<0.001) or crested ducks (P=0.014), resulting in a larger overall pH_a change in bar-headed geese (∼0.45 pH_a units

Table 2. Comparison of arterial oxygen content (Ca_{O_2}) and venous oxygen content (Cv_{O_2}) during progressive decreases in inspired partial pressure of oxygen (PI_{O_2})

	PI_{O_2} (kPa)	Ca_{O_2} (mmol l⁻¹)	Cv_{O_2} (mmol l⁻¹)
Bar-headed geese	12.20	4.7±0.3[a]	3.1±0.4[a]
	9.12	4.5±0.3[a]	3.3±0.3[a]
	7.10	3.8±0.2[b]	2.4±0.3[b]
	5.10	2.7±0.2[c]	1.8±0.3[c]
Andean geese	12.20	4.5±0.3[a]	2.6±0.3[a]
	9.12	3.9±0.4[a]	2.0±0.2[b]
	7.10	2.3±0.2[b]	1.4±0.0[c]
	5.10	1.8±0.2[c]	1.0±0.2[d]
Crested ducks	12.20	4.7±0.8[a]	2.3±0.5[a]
	9.12	3.8±0.5[b]	1.7±0.4[a,b]
	7.10	2.7±0.3[c]	1.3±0.2[b]

All values are means±s.e.m. (bar-headed geese, N=7; Andean geese, N=7; crested ducks, N=6). [a,b,c]Significant differences (P<0.05) from starting values within a species.
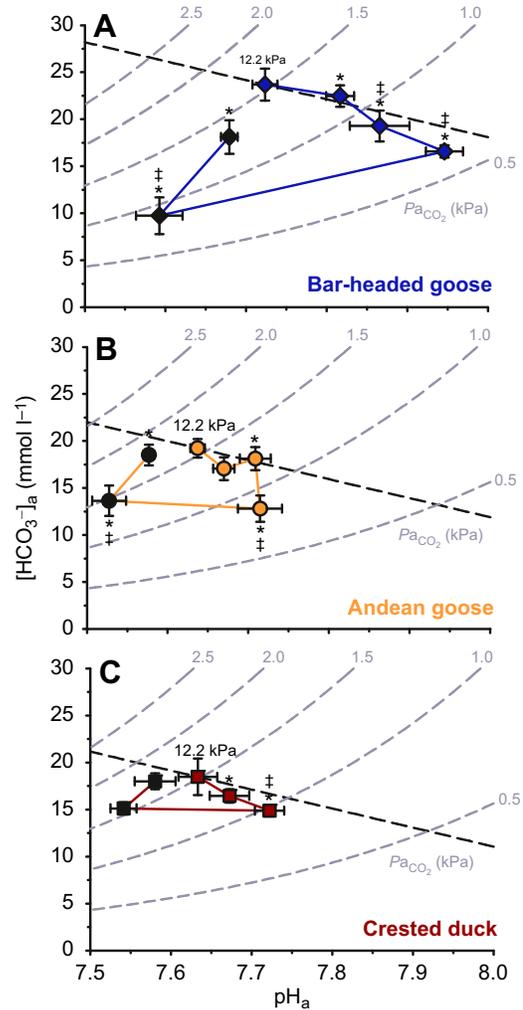

Fig. 4. Arterial acid–base status during progressive hypoxia exposure and recovery. Changes in arterial acid–base status (arterial pH, pH_a; arterial bicarbonate ion concentration, $[HCO_3^-]_a$) throughout experimental exposures in (A) bar-headed geese (N=7), (B) Andean geese (N=7) and (C) crested ducks (N=6). The point labelled 12.2 kPa represents the starting inspired partial pressure of oxygen (PI_{O_2}), with subsequent PI_{O_2} values decreasing to 9.1, 7.1 and (for the geese) 5.1 kPa. Recovery to ambient PI_{O_2} after 5 and 25 min is represented by black symbols. Grey dashed isopleths represent arterial partial pressure of CO_2 (Pa_{CO_2}; kPa) and the black dashed line is the blood buffer line (Helbecka et al., 1964). Significant differences (P<0.05) from starting values within a species are indicated by * for pH_a and ‡ for $[HCO_3^-]_a$.

versus 0.2 pH_a units in the Andean species; Fig. 4), likely as a result of the much heavier ventilation exhibited by bar-headed geese. As birds recovered in ambient conditions following hypoxia exposure, blood became acidotic in all species (Fig. 4) and plasma bicarbonate ion concentration was restored to initial levels within 25 min.

None of the birds exhibited the high [Hb] or [Hct] that typically occur in lowlanders after high-altitude acclimatization due to hypoxia-induced erythropoiesis. Hct and [Hb] were similar among bar-headed geese (Hct 31.0±2.3%; [Hb] 97.5±5.8 g l⁻¹), Andean geese (Hct 35.4±1.6%; [Hb] 108±4.1 g l⁻¹) and crested ducks (Hct 33.3±2.3%; [Hb] 103±5.9 g l⁻¹). Progressive hypoxia did not affect either Hct or [Hb], suggesting that splenic release of red blood cells was not a response to severe hypoxia (or to experimental stress) and that haemodilution did not occur with repetitive blood sampling.

Cardiovascular responses

Cardiac output (the volume of blood pumped by the heart per minute) was similar between species under starting conditions, although heart rate was higher and stroke volume lower in the crested ducks (Fig. 5). Cardiac output increased by ~2-fold during severe hypoxia exposure and to similar maximal levels among species (Fig. 5A). However, cardiac output was increased primarily by an increase in heart rate in bar-headed geese (P=0.018; Fig. 5B), but predominantly by an increase in stroke volume in the Andean species (Andean geese P=0.028 and crested ducks P=0.045; Fig. 5C,D). The large increases in cardiac output during severe hypoxia were associated with maintained mean arterial pressure (Fig. 6A) and reduced total peripheral resistance (Fig. 6B), which reached statistical significance in both Andean species and almost reached significance (P=0.055) in bar-headed geese. The blood convection requirement, the ratio of cardiac output to oxygen consumption, increased significantly in Andean geese (P=0.013) and crested ducks (P=0.012) at the most severe level of hypoxia (Fig. 7A). All three species similarly maintained tissue oxygen extraction (the amount of oxygen extracted from arterial blood; Fig. 7B), despite the fall in Ca_{O_2} during hypoxia.

DISCUSSION

Here, we provide evidence for the evolution of two strikingly divergent strategies for matching oxygen supply and demand during hypoxia in independent lineages of high-altitude waterfowl. The control processes that dictate hypoxia responses appear to have evolved in two opposing directions in the bar-headed goose – a migratory species that flies across the Himalayas – compared with the Andean goose and crested duck – non-migratory species that reside year-round at high altitude in the Andes. We suggest that long-distance high-altitude migration has favoured the evolution of a highly responsive system controlling breathing frequency, tidal volume and heart rate in hypoxia in bar-headed geese, a system that may be extremely important during occasional sojourns to extreme high altitude during migration, but may be associated with acute metabolic costs (Otis, 1954; Klabunde, 2012). Life-long high-altitude residency may have instead favoured the evolution of presumed structural (morphological) changes to the lungs and heart in the Andean species, which may accrue metabolic costs more gradually throughout development and lifelong maintenance, and should also facilitate routine flight performance at their native altitudes.
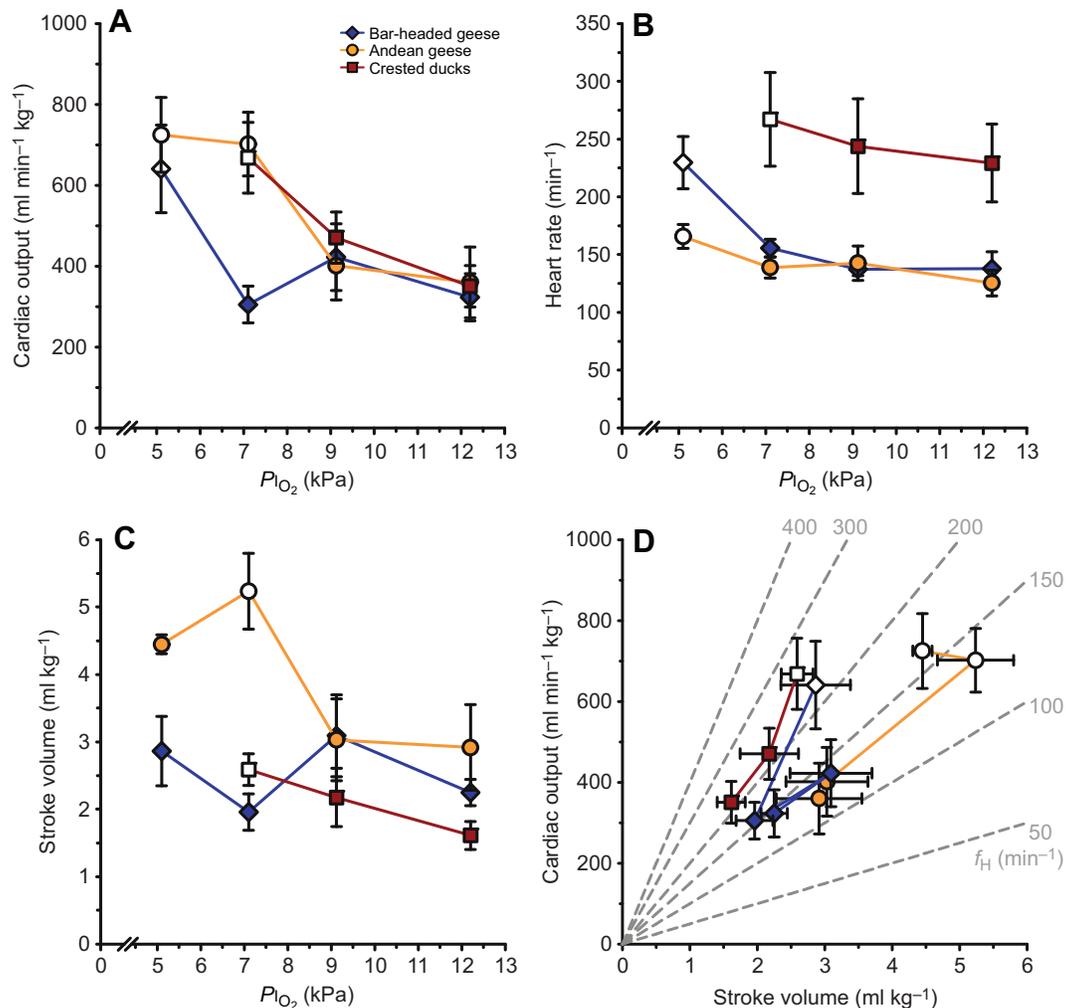

Fig. 5. Cardiovascular response patterns during progressive hypoxia. (A–C) The relationship between decreases in inspired partial pressure of oxygen ($P_{I_{O_2}}$) and (A) cardiac output, (B) heart rate and (C) stroke volume in response to progressive hypoxia. (D) Changes in cardiac output and its components, stroke volume (x-axis) and heart rate (dashed isopleths) in response to progressive hypoxia. All values represent means±s.e.m. (bar-headed geese, N=7; Andean geese, N=7; crested ducks, N=6). Significant differences (P<0.05) in cardiac output from starting values within a species are indicated by open symbols.
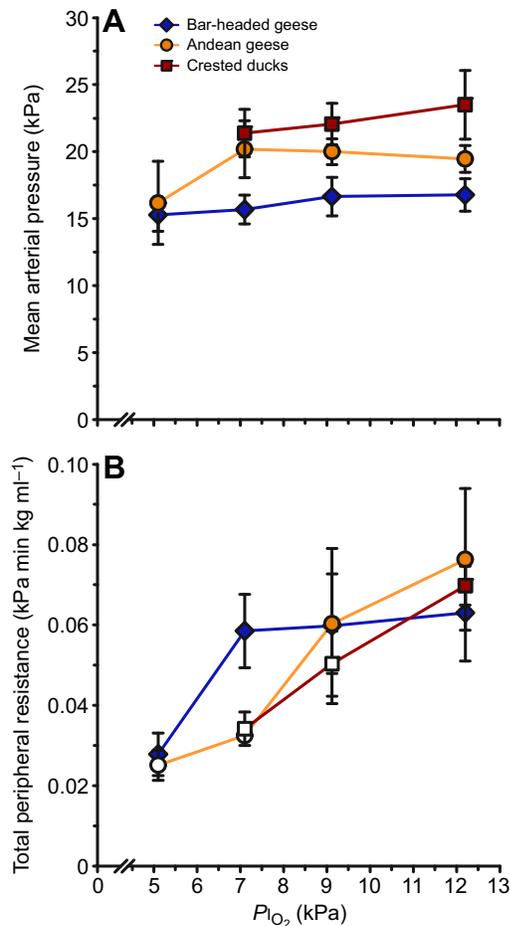
Fig. 6. Mean arterial pressure and total peripheral resistance responses during progressive hypoxia. (A) Mean arterial pressure and (B) total peripheral resistance are depicted as a function of inspired partial pressure of oxygen ($P_{I_{O_2}}$). All values represent means±s.e.m. (bar-headed geese, N=7; Andean geese, N=7; crested ducks, N=6). Significant differences (P<0.05) from starting values within a species are indicated by open symbols.

Fig. 7. Cardiovascular responses to progressive hypoxia. Changes in (A) the blood convection requirement and (B) tissue oxygen extraction during progressive hypoxia. All values represent means±s.e.m. (bar-headed geese, N=7; Andean geese, N=7; crested ducks, N=6). Significant differences (P<0.05) from starting values within a species are indicated by open symbols.

Bar-headed geese ventilate heavily to deliver more oxygen to the gas exchange surfaces, consistent with previous studies (Black and Tenney, 1980a; Scott and Milsom, 2007; Lague et al., 2016), and in stark contrast to the modest hypoxic ventilatory response of the Andean species. The robust ventilatory response of bar-headed geese produced a respiratory alkalosis that likely enhanced blood-oxygen loading via the Bohr shift, given that bar-headed geese exhibit a normal Bohr effect (Black and Tenney, 1980a; Meir and Milsom, 2013). This may explain why bar-headed geese maintained similar Ca_{O_2} to that of the other species *in vivo*, despite having a lower intrinsic Hb–oxygen affinity than that of Andean geese (Black and Tenney, 1980a; McCracken et al., 2010). In contrast, the Andean species appear to increase lung oxygen extraction in hypoxia, precluding the need for a robust ventilatory response to hypoxia. This observation was based on calculations of the lung oxygen extraction that would be necessary to support the observed metabolic rates at the measured level of total ventilation, and suggest that an extremely high percentage of the inspired oxygen can be extracted by the lungs of the Andean species (Fig. 1). It is possible that our assumption that RQ equalled 1.0 throughout the experiment led to an over-estimation of lung oxygen extraction, but we expect that this effect was minor. Although RQ can range from <0.7 to 1.0 in birds, it has been shown that acute hypoxic exposure does not
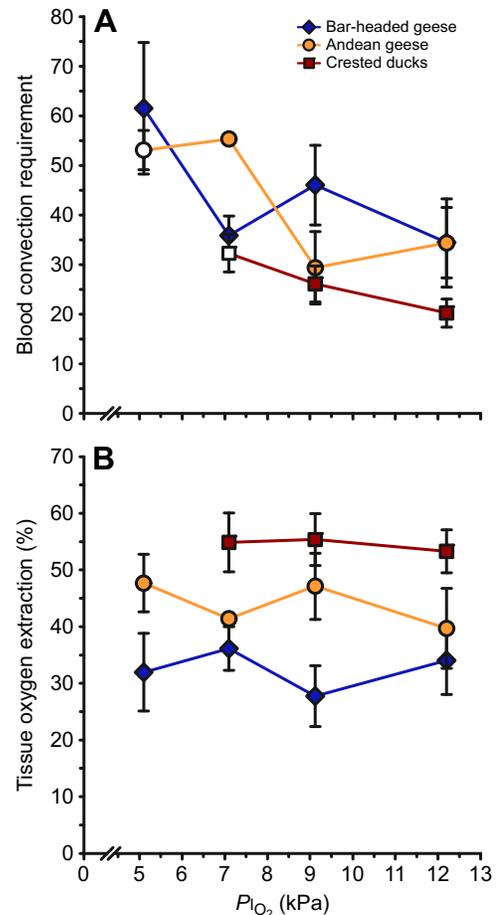
change RQ in bar-headed geese (Hawkes et al., 2014). If RQ had exceeded 1.0 in the Andean species (an unlikely possibility given their low rates of ventilation), then our oxygen consumption rate values would be overestimated by 3.5–11.5% (based on a starting RQ of 1.0–0.7, respectively, in normoxic conditions) (Withers, 1977). Therefore, lung oxygen extraction could have been slightly less than that reported here, but still much higher in the Andean species than in bar-headed geese. Although the basis of the potentially high lung oxygen extraction in the Andean species is presently unknown, and it will be necessary to confirm these findings with future direct measurements of pulmonary function, we hypothesize that high oxygen extraction could be explained by a combination of functional factors (e.g. higher oxygen affinity of Andean geese, and increased pulmonary capillary recruitment occurring with the decreased ventilation–perfusion ratio present during hypoxia) working in tandem with morphological enhancements (e.g. increased lung surface area, increased capillarity and/or decreased barrier thickness) (Maina et al., 2017).

All species also appear to increase cardiac output in hypoxia, which would help ensure sufficient oxygen delivery from the lungs to the tissues, but our data suggest that there were different strategies for increasing cardiac output between species. Bar-headed geese increase cardiac output by increasing heart rate, whereas the Andean species appear to do so primarily by increasing stroke volume.

These findings should be treated with some caution, given that cardiac output was calculated and not measured directly (see Materials and methods). We were unable to confirm exact placement of venous catheters post-mortem in all individuals, so there could have been some variation in our Cv_{O_2} measurements that affected our calculations of cardiac output. However, this is likely a minor issue, because our Cv_{O_2} data exhibited similar variance to our Ca_{O_2} data (for which catheter placement would have had no effect) (Table 2), and we would have expected the variance to be greater if catheter placement had had a significant influence on Cv_{O_2}. Nevertheless, our data indicate that the cardiovascular responses to hypoxia also differ between species.

The divergent strategies of hypoxia tolerance employed between bird species have intriguing similarities with those reported for high-altitude Tibetan and Andean humans. High-altitude Tibetan humans have high levels of resting ventilation and respond to hypoxia with a robust ventilatory response (Beall et al., 1998; Beall, 2002; Wu and Kayser, 2006), much like the Himalayan bar-headed geese. In contrast, Andean high-altitude humans have a blunted ventilatory response to hypoxia and rely predominantly on enhanced pulmonary oxygen diffusing capacity to maintain oxygen uptake (Beall et al., 1998; Beall, 2007; Winslow et al., 1989), in addition to an enhanced blood Hct and [Hb] (Beall, 2007). It has been argued that the response of Andean high-altitude human populations is an off-target and maladaptive response to chronic hypoxia because increasing Hct also increases blood viscosity, which exacerbates the pulmonary hypertension resulting from pulmonary artery hypoxic vasoconstriction and contributes to chronic mountain sickness (Monge et al., 1992; Simonson, 2015; Dempsey and Morgan, 2015). Recent evidence even suggests that a high blood [Hb] at high altitudes reduces oxygen transport capacity during exercise, likely in part because the increase in Ca_{O_2} is more than offset by a viscosity-induced reduction in cardiac output (Simonson et al., 2015). The increase in Hb–oxygen affinity in the avian Andean species (Weber et al., 1993; McCracken et al., 2010), a high-altitude adaptation that has not arisen in Andean humans, likely helps improve Ca_{O_2} without increasing blood viscosity. In addition, there is no indication that the pulmonary vasculature constricts in response to hypoxia in birds (Faraci et al., 1984; West et al., 2007). Andean birds therefore appear to have evolved some of the beneficial strategies for matching oxygen supply and demand in hypoxia that are employed by Andean humans (e.g. increased lung oxygen extraction) without adopting those that are believed to be maladaptive. The similarly divergent paths taken between high-altitude lineages in the South America and Asia could suggest that the selective pressures at high altitudes may differ between different parts of the world, leading to different functional outcomes of high-altitude adaptation.

References
Beall, C. M. (2002). Tibetan and Andean contrasts in adaptation to high-altitude hypoxia. In *Oxygen Sensing: Molecule to Man* (ed. S. Lahiri, N. R. Prabhakar and R. E. ForsterII), pp. 63-74. New York: Kluwer Academic Publishers.

Beall, C. M. (2007). Two routes to functional adaptation: Tibetan and Andean high-altitude natives. *PNAS* **104**, 8655-8660.

Beall, C. M., Strohl, K. P., Blangero, J., Williams-Blangero, S., Almasy, L. A., Decker, M. J., Worthman, C. M., Goldstein, M. C., Vargas, E., Villena, M. et al. (1997). Ventilation and hypoxic ventilatory response of Tibetan and Aymara high altitude natives. *Am. J. Phys. Anthropol.* **104**, 427-447.

Beall, C. M., Brittenham, G. M., Strohl, K. P., Blangero, J., Williams-Blangero, S., Goldstein, M. C., Decker, M. J., Vargas, E., Villena, M., Soria, R. et al. (1998). Hemoglobin concentration of high-altitude Tibetans and Bolivian Aymara. *Am. J. Phys. Anthropol.* **106**, 385-400.

Bech, C. and Nomoto, S. (1982). Cardiovascular changes associated with treadmill running in the Pekin duck. *J. Exp. Biol.* **97**, 345-358.

Bishop, C. M., Spivey, R. J., Hawkes, L. A., Batbayar, N., Chua, B., Frappell, P. B., Milsom, W. K., Natsagdorj, T., Newman, S. H., Scott, G. R. et al. (2015). The roller coaster flight strategy of bar-headed geese conserves energy during Himalayan migrations. *Science* **347**, 250-254.

Black, C. P. and Tenney, S. M. (1980a). Oxygen transport during progressive hypoxia in high-altitude and sea-level waterfowl. *Respir. Physiol.* **39**, 217-239.

Black, C. P. and Tenney, S. M. (1980b). Pulmonary hemodynamic responses to acute and chronic hypoxia in two waterfowl species. *Comp. Biochem. Physiol. A* **67A**, 291-293.

Bouverot, P. (1985). General introduction. In *Adaptation to Altitude-Hypoxia in Vertebrates*, pp. 1-18. Berlin: Springer-Verlag.

Brutsaert, T. D., Araoz, M., Soria, R., Spielvogel, H. and Haas, J. D. (2000). Higher arterial oxygen saturation during submaximal exercise in Bolivian Aymara compared to European sojourners and Europeans born and raised at high altitude. *Am. J. Phys. Anthropol.* **113**, 169-181.

Calder, W. A. (1968). Respiratory and heart rates of birds at rest. *Condor* **70**, 358-365.

Dejours, P. (1975). Appendix. In *Principles of Comparative Respiratory Physiology*, pp. 215-223. New York: Elsevier.

Dempsey, J. A. and Morgan, B. J. (2015). Humans in hypoxia: a conspiracy of maladaptation?! *Physiology* **30**, 304-316.

Faraci, F., Kilgore, D. and Fedde, M. R. (1984). Attenuated pulmonary pressor response to hypoxia in bar-headed geese. *Am. J. Physiol.* **247**, R402-R403.

Fjeldså, J. and Krabbe, N. (1990). *Birds of the High Andes*. Copenhagen: Zoological Museum, University of Copenhagen.

Fleisch, A. (1925). Der Pneumotachograph; ein Apparat zur Geschwindigkeitsregistrierung der Atemluft. *Pflugers Arch.* **209**, 713-722.

Grubb, B. R. (1983). Allometric relations of cardiovascular function in birds. *Am. J. Physiol.* **245**, H567-H572.

Harter, T. S., Reichert, M., Brauner, C. J. and Milsom, W. K. (2015). Validation of the i-STAT and HemoCue systems for the analysis of blood parameters in the bar-headed goose, *Anser indicus. Conserv. Physiol.* **3**, cov021.

Hawkes, L. A., Balachandran, S., Batbayar, N., Butler, P. J., Frappell, P. B., Milsom, W. K., Tseveenmyadag, N., Newman, S. H., Scott, G. R., Sathiyaselvam, P. et al. (2011). The trans-Himalayan flights of bar-headed geese (*Anser indicus*). *PNAS* **108**, 9516-9519.

Hawkes, L. A., Butler, P. J., Frappell, P. B., Meir, J. U., Milsom, W. K., Scott, G. R. and Bishop, C. M. (2014). Maximum running speed of captive bar-headed geese is unaffected by severe hypoxia. *PLoS ONE* **9**, e94015.

Helbecka, N. V. L., Casterline, J. L., Smith, C. J. and Shaffner, C. S. (1964). Investigation of plasma carbonic acid pK of the chicken. *Poult. Sci.* **43**, 138-144.

Klabunde, R. E. (2012). Cardiac function. In *Cardiovascular Physiology Concepts*, pp. 60-92. Baltimore: Lippincott Williams & Wilkins.

Lague, S. L., Chua, B., Farrell, A. P., Wang, Y. and Milsom, W. K. (2016). Altitude matters: differences in cardiovascular and respiratory responses to hypoxia in bar-headed geese reared at high and low altitudes. *J. Exp. Biol.* **219**, 1974-1984.

Maina, J. N., McCraken, K. G., Chua, B., York, J. M. and Milsom, W. K. (2017). Morphological and morphometric specializations of the lung of the Andean goose, Chloephaga melanoptera: a lifelong high-altitude resident. *PLoS ONE* **12**, e0174395.

Meir, J. U. and Milsom, W. K. (2013). High thermal sensitivity of blood enhances oxygen delivery in the high-flying bar-headed goose. *J. Exp. Biol.* **216**, 2172-2175.

McCracken, K. G., Barger, C. P. and Sorenson, M. D. (2010). Phylogenetic and structural analysis of the HbA (αA/βA) and HbD (αD/βA) hemoglobin genes in two high-altitude waterfowl from the Himalayas and the Andes: Bar-headed goose (*Anser indicus*) and Andean goose (*Chloephaga melanoptera*). *Mol. Phylogenet. Evol.* **56**, 649-658.

Monge, C. C., Arregui, A. and León-Verlarde, F. (1992). Pathophysiology and epidemiology of chronic mountain sickness. *Intern. J. Sports Med.* **13**, S79-S81.

Natarajan, C., Projecto-Garcia, J., Moriyama, H., Weber, R. E., Muñoz-Fuentes, V., Green, A. J., Kopuchian, C., Tubaro, P. L., Alza, L., Bulgarella, M. et al. (2015). Convergent evolution of hemoglobin function in high-altitude Andean waterfowl involves limited parallelism at the molecular sequence level. *PLoS Genet.* **11**, e1005681.

Otis, A. B. (1954). The work of breathing. *Physiol. Rev.* **34**, 449-458.

Scheid, P. (1990). Avian respiratory system and gas exchange. In *Hypoxia: The Adaptations* (ed. J. Sutton, G. Coates and J. E. Remmers), pp. 4-7. Toronto: B.C. Decker.

Scott, G. R. (2011). Elevated performance: the unique physiology of birds that fly at high altitudes. *J. Exp. Biol.* **214**, 2455-2462.

Scott, G. R. and Milsom, W. K. (2007). Control of breathing and adaptation to high altitude in the bar-headed goose. *Am. J. Physiol.* **293**, R379-C389.

Scott, G. R., Egginton, S., Richards, J. G. and Milsom, W. K. (2009). Evolution of muscle phenotype for extreme high altitude flight in the bar-headed goose. *Proc. R. Soc. B* **276**, 3645-3653.

Scott, G. R., Hawkes, L. A., Frappell, P. B., Butler, P. J., Bishop, C. M. and Milsom, W. K. (2015). How bar-headed geese fly over the Himalayas. *Physiology* **30**, 107-115.

Shams, H. and Scheid, P. (1989). Efficiency of parabronchial gas exchange in deep hypoxia: measurements in the resting duck. *Respir. Physiol.* **77**, 135-146.

Simonson, T. S. (2015). Altitude adaptation: a glimpse through various lenses. *High Alt. Med. Biol.* **16**, 125-137.

Simonson, T. S., Huff, C. D., Witherspoon, D. J., Prchal, J. T. and Jorde, L. B. (2015). Adaptive genetic changes related to haemoglobin concentration in native high-altitude Tibetans. *Exp. Physiol.* **100**, 1263-1268.

Smith, F. M., West, N. H. and Jones, D. R. (2000). The cardiovascular system. In *Sturkie's Avian Physiology* (ed. G. C. Whittow), pp. 141-232. San Diego: Academic Press.

Tucker, V. (1967). Method for oxygen content and dissociation curves on microliter blood samples. *J. Appl. Physiol.* **23**, 410-414.

Weber, R., Jessen, T., Malte, H. and Tame, J. (1993). Mutant hemoglobins (alpha 119-Ala and beta 55-Ser): functions related to high-altitude respiration in geese. *J. Appl. Physiol.* **75**, 2646-2655.

West, J. B., Watson, R. R. and Fu, Z. (2007). Major differences in the pulmonary circulation between birds and mammals. *Respir. Physiol. Neurobiol.* **157**, 382-390.

Winslow, R. M., Chapman, K. W., Gibson, C. C., Samaja, M., Monge-C, C., Goldwasser, E., Sherpa, M., Blume, F. D. and Santolaya, R. (1989). Different hematologic responses to hypoxia in Sherpas and Quechua Indians. *J. Appl. Physiol.* **66**, 1561-1569.

Withers, P. C. (1977). Measurement of V_{O_2}, V_{CO_2}, and evaporative water loss with a flow-through mask. *J. Appl. Physiol.* **42**, 120-123.

Wu, T. and Kayser, B. (2006). High altitude adaptation in Tibetans. *High Alt. Med. Biol.* **7**, 193-208.