© 2016. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2016) 219, 1974-1984 doi:10.1242/jeb.132431

RESEARCH ARTICLE

Altitude matters: differences in cardiovascular and respiratory responses to hypoxia in bar-headed geese reared at high and low altitudes

Sabine L. Lague[1,*], Beverly Chua[1], Anthony P. Farrell[1,2], Yuxiang Wang[3] and William K. Milsom[1]

ABSTRACT

Bar-headed geese (*Anser indicus*) fly at high altitudes during their migration across the Himalayas and Tibetan plateau. However, we know relatively little about whether rearing at high altitude (i.e. phenotypic plasticity) facilitates this impressive feat because most of what is known about their physiology comes from studies performed at sea level. To provide this information, a comprehensive analysis of metabolic, cardiovascular and ventilatory responses to progressive decreases in the equivalent fractional composition of inspired oxygen ($F_{I_{O_2}}$: 0.21, 0.12, 0.09, 0.07 and 0.05) was made on bar-headed geese reared at either high altitude (3200 m) or low altitude (0 m) and on barnacle geese (*Branta leucopsis*), a low-altitude migrating species, reared at low altitude (0 m). Bar-headed geese reared at high altitude exhibited lower metabolic rates and a modestly increased hypoxic ventilatory response compared with low-altitude-reared bar-headed geese. Although the *in vivo* oxygen equilibrium curves and blood-oxygen carrying capacity did not differ between the two bar-headed goose study groups, the blood-oxygen carrying capacity was higher than that of barnacle geese. Resting cardiac output also did not differ between groups and increased at least twofold during progressive hypoxia, initially as a result of increases in stroke volume. However, cardiac output increased at a higher $F_{I_{O_2}}$ threshold in bar-headed geese raised at high altitude. Thus, bar-headed geese reared at high altitude exhibited a reduced oxygen demand at rest and a modest but significant increase in oxygen uptake and delivery during progressive hypoxia compared with bar-headed geese reared at low altitude.

KEY WORDS: *Anser indicus*, *Branta leucopsis*, Barnacle goose, Ventilation, Cardiac output, Metabolic rate, Oxygen uptake, Stroke volume

INTRODUCTION

Birds exhibit enhancements at each step of their O_2 transport cascade that help to support the high flux of O_2 required for flight and endothermy (Scott, 2011). This cascade describes the flow of O_2 from the atmosphere to the mitochondria in vertebrates and the steps include ventilation, pulmonary O_2 diffusion, perfusion and tissue O_2 diffusion. Bar-headed geese (*Anser indicus*), which migrate biannually over the Himalayan mountain range (Bishop et al., 2015; Hawkes et al., 2011; Scott et al., 2015), exhibit further adaptations at each level of this transport cascade (Black and Tenney, 1980; Meir and Milsom, 2013; Scott and Milsom, 2006, 2007; Scott, 2011; Scott et al., 2015; Weber et al., 1993). While providing evidence of physiological differences between bar-headed geese and closely related low-altitude species, all studies to date have been conducted on groups of bar-headed geese born and raised for generations at sea level (Black and Tenney, 1980; Fedde et al., 1989; Hawkes et al., 2014; Scott and Milsom, 2007). Thus, relatively little is known about the influence of phenotypic plasticity (i.e. acclimatization) and developmental plasticity on the physiology of this species.

Hypoxic responses vary depending on when during an animal's development it is exposed to hypoxia and the duration of the exposure. In chickens, hypoxic exposure had no reported developmental effects when it occurred early in development or for an acute duration (Ferner and Mortola, 2009). When exposure to hypoxia occurred during the entire duration of incubation, or even during the final week, however, the hypoxic ventilatory response (HVR) of the chicks was blunted. This was a result of reduced ventilatory chemosensitivity (Ferner and Mortola, 2009; Mortola, 2011; Szdzuy and Mortola, 2007). Sustained hypoxic exposure in low-altitude birds also decreased whole animal oxygen consumption rate ($\dot{V}_{O_2}$) and growth rate (Mortola, 2011). However, embryos of some birds successfully hatch with normal growth rates and $\dot{V}_{O_2}$ at altitudes of 4000–6500 m (Carey et al., 1982; León-Velarde and Monge-C, 2004). For example, the high-altitude migrating bar-headed goose maintained $\dot{V}_{O_2}$ as an embryo when exposed acutely to extreme ambient hypoxia (11.7 kPa) (Snyder et al., 1982). Thus, although hypoxia exposure during development can alter physiological responses in birds, some high-altitude bird species have adapted to mitigate these effects.

In adult animals, many rapid physiological changes occur minutes to hours after acute hypoxic exposure and can be modified during chronic acclimatization (Ivy and Scott, 2015; Powell et al., 1998). Black and Tenney measured changes in $\dot{V}_{O_2}$, total ventilation ($\dot{V}_R$) and cardiac output ($\dot{Q}$) during progressive hypoxic exposure in bar-headed geese following short-term (4 week) acclimation to simulated altitude (5640 m) (Black and Tenney, 1980). The acclimated bar-headed geese did not become polycythemic, a trait characteristic of other species endemic to high-altitude regions. They also displayed higher resting $\dot{V}_{O_2}$, $\dot{V}_R$ and $\dot{Q}$ under ambient conditions, as well as greater increases in $\dot{V}_R$ and $\dot{Q}$ during exposure to progressive hypoxia (Black and Tenney, 1980). Thus, the HVR and hypoxic cardiovascular response of bar-headed geese can be affected by short-term high-altitude acclimation.

In the present study, we extended this work by examining the effects of high-altitude rearing and development on the physiological responses to hypoxia in bar-headed geese. This is

[1]Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4. [2]Faculty of Land and Food Systems, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4. [3]Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

*Author for correspondence (lague@zoology.ubc.ca)

S.L.L., 0000-0002-8724-7297

Received 18 September 2015; Accepted 6 April 2016

Journal of Experimental Biology

Journal of Experimental Biology (2016) 219, 1974-1984 doi:10.1242/jeb.132431

List of symbols and abbreviations

BTPS	body temperature and pressure saturated
Ca_{O_2}	arterial oxygen content
Cv_{O_2}	venous oxygen content
$F_{E_{O_2}}$	fractional oxygen composition of expired gas of the bird
$F_{EC_{O_2}}$	fractional oxygen composition of expired gas of the chamber
$F_{I_{O_2}}$	fractional oxygen composition of inspired gas
HAR	high-altitude-reared
Hb	hemoglobin
[Hb]	hemoglobin concentration
Hct	hematocrit
$[HCO_3^-]_a$	arterial bicarbonate ion concentration
HVR	hypoxic ventilatory response
LAR	low-altitude-reared
Pa_{CO_2}	arterial partial pressure of carbon dioxide
Pa_{O_2}	arterial partial pressure of oxygen
pH_a	arterial pH
$\dot{Q}$	cardiac output
RQ	respiratory quotient
STPD	standard temperature and pressure and dry
$\dot{V}_{O_2}$	whole animal oxygen consumption rate
$\dot{V}_C$	flow rate through chamber
$\dot{V}_R$	total ventilation

significant, considering that the effects of pre- or postnatal hypoxic exposure can differ significantly, and persist throughout adult life (Bavis, 2005; Ivy and Scott, 2015). Our primary objective, therefore, was to compare the changes in the convective steps in the O_2 transport cascade, ventilation and circulation, of low-altitude-reared (LAR) bar-headed geese to a group of wild high-altitude-reared (HAR) bar-headed geese during short-term progressive hypoxic exposure. These responses were compared with those of LAR barnacle geese (*Branta leucopsis*), a member of a closely related genus that also migrates, but only at low altitude. We predicted that, because of lifelong exposure to high altitude, HAR bar-headed geese would show further enhancements in the overall magnitude of $\dot{V}_R$ and $\dot{Q}$ compared with those previously reported during short-term high altitude acclimation of this species (Black and Tenney, 1980). In addition, because bar-headed geese are known to be capable of large increases in $\dot{V}_R$ during hypoxic exposure (Black and Tenney, 1980; Scott and Milsom, 2007), we predict that $\dot{V}_R$ would be greatest in the HAR bar-headed geese and lowest in the barnacle geese.

MATERIALS AND METHODS

Animals

The experiments on the LAR geese were performed at the University of British Columbia, where the geese were housed at the Centre for Comparative Medicine. Cardiovascular measurements were made on 6 cannulated bar-headed geese (*Anser indicus* Latham 1790) (2.5±0.2 kg) and respiratory measurements were made on 5 non-cannulated bar-headed geese (2.4±0.1 kg). All cardiorespiratory measurements were made on 7 cannulated barnacle geese (*Branta leucopsis* Bechstein 1803) (2.5±0.2 kg). Cardiorespiratory measurements were also obtained from 5 cannulated HAR bar-headed geese (2.1±0.1 kg) that were born in the wild at 3200 m at Lake Qinghai, China and reared in captivity for at least 1 year at the lake. All experimental animals were fed similar diets, housed in outdoor pens under natural conditions and experienced similar levels of (in)activity. The HAR bar-headed geese, however, were born and reared in hypobaric hypoxia. All experimental procedures were conducted according to guidelines approved by the Animal Care Committee at the University of British Columbia under the guidelines of the Canadian Council on Animal Care.

Surgical procedures

For cardiovascular measurements, surgery was conducted under general and local anesthesia 1 day before the hypoxic exposure. All geese were first weighed, gently restrained and induced with isoflurane (4%) supplemented with O_2 (100%) by facemask prior to intubation. General anesthesia 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. Geese were recovered for at least 24 h prior to experimentation.

Experimental protocol

Each goose was placed in a flexible cradle that permitted unrestricted breathing. Its head was placed in an opaque Plexiglass chamber large enough to accommodate free movement of the neck and head, sealed around the neck with a flexible latex collar and supported by the cradle. Geese in the experimental apparatus were allowed 60–90 min to adjust to their surroundings. Then, air with varying equivalent fractional compositions of inspired O_2 ($F_{I_{O_2}}$) was delivered at a flow rate ($\dot{V}_C$) through the box ranging between 5 and 10 l min^{-1}. Changes in $F_{I_{O_2}}$ were produced by mixing nitrogen and air through a series of calibrated rotameters. Birds were exposed to 25 min step reductions in equivalent $F_{I_{O_2}}$: ambient [0.21 at 0 m or 0.134 at 3200 m], 0.12, 0.09 and 0.07. For the respiratory trials, birds were exposed further to 0.05 $F_{I_{O_2}}$. A 25 min recovery at ambient $F_{I_{O_2}}$ followed the hypoxic exposures.

Measurements

Whole animal $\dot{V}_{O_2}$ was calculated from $\dot{V}_C$, $F_{I_{O_2}}$ and the fractional O_2 composition of expired gas from the chamber ($F_{EC_{O_2}}$), which were directly measured by a gas analyzer (Sable Systems, Las Vegas, NV, USA). Water vapour was removed from the gas prior to analysis. Tidal volume and breathing frequency were measured from the head mask outflow using a pneumotachograph connected to a differential pressure transducer (Validyne, Northridge, CA, USA).

Mean arterial pressure and all respiratory variables were recorded to a computer using PowerLab data acquisition software (ADInstruments, Colorado Springs, CO, USA). Arterial blood pressure was continuously monitored throughout using a pressure transducer (Deltran, Utah Medical Products, Midvale, USA) connected to the brachial artery cannula. Strategic sampling of arterial and venous blood (0.4 ml per sample) occurred 15 min after exposure to each $F_{I_{O_2}}$, as well as after 5 and 25 min in recovery. Any blood remaining after analysis was returned to the bird. Blood samples were immediately analyzed for partial pressures of O_2 and CO_2, O_2 content, Hb concentration ([Hb]), hematocrit (Hct), arterial pH (pH_a) and plasma ions including HCO_3^-. Arterial O_2 content (Ca_{O_2}; mmol l^{-1}) and venous O_2 content (Cv_{O_2}; mmol l^{-1}) were determined at 41°C using the Tucker method (Tucker, 1967) with a FireSting O_2 probe (PyroScience, Aachen, Germany). The O_2 probe was calibrated with 0% O_2 (3 g l^{-1} Na_2SO_3; Sigma-Aldrich) and water saturated with ambient air (21% at sea level and 12% at 3200 m) prior to each experiment. [Hb] (g dl^{-1}), Hct (%), arterial bicarbonate ion concentration ($[HCO_3^-]_a$; mmol l^{-1}), Pa_{O_2} (kPa), arterial partial pressure of carbon dioxide (Pa_{CO_2}; kPa), and arterial pH (pH_a) were analyzed from arterial blood at 41°C using CG8+

Journal of Experimental Biology (2016) 219, 1974-1984 doi:10.1242/jeb.132431

cartridges with the i-STAT VetScan Analyzer (Abaxis, Union City, CA, USA). All i-STAT values were corrected according to Harter et al. (2015). $[HCO_3^-]_a$ was calculated using the Henderson–Hasselbach 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; Scott and Milsom, 2007).

Data and statistical analysis

With the exception of the blood variables, all data were acquired and analyzed using the PowerLab data acquisition and analysis software (ADInstruments, Colorado Springs, CO, USA) at a sampling frequency of 1000 Hz per channel. Mean values were derived for each variable for a 1–2 min period before each blood sample (e.g. after 12–15 min of each hypoxic $F_{I_{O_2}}$ exposure, and after 3–5 min and 22–25 min of the normoxic recovery). Ca_{O_2} and Cv_{O_2} were acquired using software designed for the FireSting O_2 probes.

The following variables were either measured directly or used to calculate additional variables. Tidal volume (ml kg^{-1}) and breathing frequency (min^{-1}) were derived from the integrated differential pressure signal and $\dot{V}_R$ (ml min^{-1} kg^{-1}) was calculated as their product. $\dot{V}_{O_2}$ (ml min^{-1} kg^{-1}) was calculated as:

$$\dot{V}_{O_2} = \frac{\dot{V}_C \times (F_{I_{O_2}} - F_{EC_{O_2}})}{M_b}, \quad (1)$$

where M_b is body mass (kg). Water vapour was removed prior to gas analysis (Withers, 1977) and the respiratory quotient (RQ) was assumed to be 1.0. It has been shown that RQ does not change with acute hypoxic exposure in bar-headed geese (Hawkes et al., 2014) and is unaffected by prolonged hypoxic exposure in deer mice (Cheviron et al., 2012; McClelland et al., 1998). Thus, while RQ in birds can range from <0.7 to 1.0 and our calculations ignore this variation, they use the same value throughout, thus allowing for comparison without affecting our overall conclusions (Scott and Milsom, 2007). Air convection requirement was calculated as the quotient of $\dot{V}_R$ and $\dot{V}_{O_2}$. Lung O_2 extraction (%) was calculated as:

$$\text{Lung } O_2 \text{ extraction} = \left(\frac{F_{I_{O_2}} - F_{E_{O_2}}}{F_{I_{O_2}}}\right) \times 100, \quad (2)$$

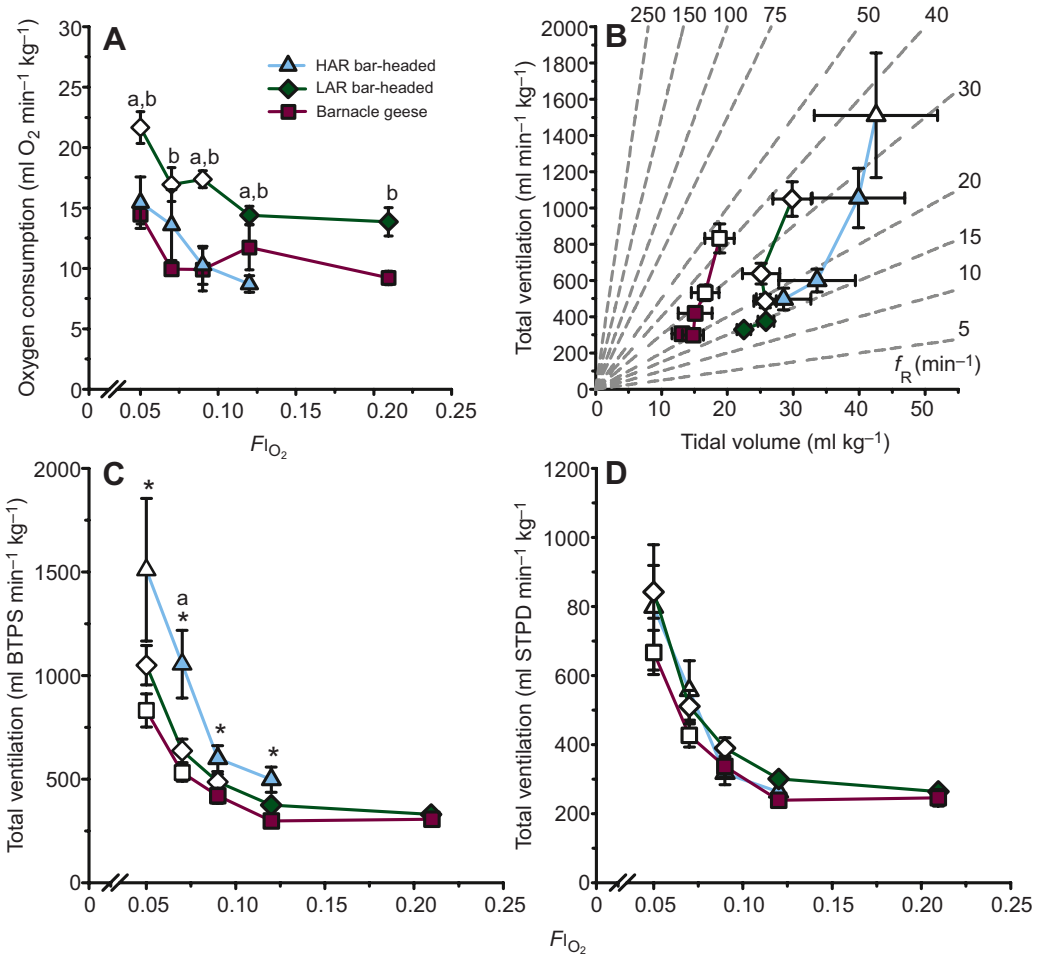

Fig. 1. Oxygen consumption is maintained throughout hypoxia exposure by increases in total ventilation. (A) Oxygen consumption (STPD) plotted as a function of the fractional oxygen composition of inspired gas ($F_{I_{O_2}}$) in all groups. (B) Hey plot depicting the breathing patterns of all groups. For any level of total ventilation, bar-headed geese had a higher tidal volume and lower breathing frequency (right-shifted curve). Dashed lines represent breathing frequency (f_R) isopleths. (C) Total ventilation as a function of the fractional oxygen composition of inspired gas ($F_{I_{O_2}}$) in all groups in BTPS. (D) Total ventilation as a function of the fractional oxygen composition of inspired gas ($F_{I_{O_2}}$) in all groups in STPD. Values represent means±s.e.m. (blue: high-altitude-reared bar-headed geese, N=5; green: low-altitude-reared bar-headed geese, N=5; purple: barnacle geese, N=7). Significant differences (P<0.05) in the y-axis variable from values during exposure to ambient air within a species are indicated by open symbols and determined by one-way repeated measured ANOVA. Significant differences (P<0.05) in the y-axis variable (A,C and D) between species are determined by two-way repeated measures ANOVA and indicated by different symbols: *HAR bar-headed geese versus barnacle geese; [a]HAR bar-headed geese versus LAR bar-headed geese; and [b]LAR bar-headed geese versus barnacle geese.

Journal of Experimental Biology

Journal of Experimental Biology (2016) 219, 1974-1984 doi:10.1242/jeb.132431

where the calculated fractional expired level of O_2 of the bird ($F_{E_{O_2}}$) was calculated as:

$$F_{E_{O_2}} = \frac{(\dot{V}_R \times F_{I_{O_2}}) - \dot{V}_{O_2}}{\dot{V}_R}. \quad (3)$$

$\dot{V}_{O_2}$, air convection requirement and lung O_2 extraction were reported in terms of standard temperature and pressure and dry (STPD). Tidal volume was reported in terms of body temperature and pressure saturated (BTPS), assuming a constant body temperature of 41°C and taking into account changes in barometric pressure and air density at altitude (Dejours, 1975). $\dot{V}_R$ was reported in both BTPS and STPD for comparison.

Regarding cardiovascular measurements, heart rate (min^{-1}) was calculated from the peaks in the pulsatile arterial blood pressure trace. Mean arterial pressure (kPa) was calculated as the sum of diastolic pressure plus 1/3 pulse pressure. $\dot{Q}$ (ml min^{-1} kg^{-1}) was calculated from the Fick equation given known values of $\dot{V}_{O_2}$ and Ca_{O_2}–Cv_{O_2}. Stroke volume (ml kg^{-1}) was calculated as the quotient of $\dot{Q}$ and heart rate. Total peripheral resistance (kPa min kg ml^{-1}) was calculated as the quotient of mean arterial pressure and $\dot{Q}$ (Bech and Nomoto, 1982). Blood convection requirement was calculated as the quotient of $\dot{Q}$ and $\dot{V}_{O_2}$. Tissue O_2 delivery was calculated as the product of $\dot{Q}$ and Ca_{O_2} (ml^{-1} min^{-1} kg^{-1}). Tissue O_2 extraction (%) was calculated as:

$$\text{Tissue } O_2 \text{ extraction} = \frac{(Ca_{O_2} - Cv_{O_2})}{Ca_{O_2}} \times 100. \quad (4)$$

We corrected the Hct and [Hb] data collected by the i-STAT VetScan Analyzer for all groups based on the calibrations derived for bar-headed geese in Harter et al. (2015).

Data are presented as means±s.e.m. unless stated otherwise. Within each species, all data were analyzed using one-way repeated measures analysis of variance (ANOVA) and Holm–Šidák *post hoc* tests. Comparisons between each species were made using two-way (species and $F_{I_{O_2}}$) repeated measures ANOVA and Holm–Šidák *post hoc* tests within each $F_{I_{O_2}}$. For statistical comparisons, $P<0.05$ was used to determine statistical significance. Variables analyzed with a one-way repeated measures ANOVA that did not meet assumptions for either normality or equal variance in barnacle geese were transformed with $x'=\ln(x)$ for $\dot{Q}$, lung O_2 extraction, and blood convection requirement and with $x'=x^2$ for tidal volume. Similarly, variables were transformed when they did not meet assumptions for either normality or equal variance analyzed for a two-way repeated measures ANOVA (i.e. $x'=\ln(x)$ and $x'=1/(1-x)$ for $\dot{V}_R$, tidal volume and Pa_{O_2}). Student's *t*-tests were used to compare Hct and [Hb] prior to and following the experiment to ensure that no blood dilution had been incurred throughout the experiment. Statistical analyses were carried out using SigmaStat (version 3.0; Systat Software).

RESULTS

Metabolic response

Both bar-headed and barnacle geese maintained $\dot{V}_{O_2}$ during progressive hypoxia (Fig. 1A), with $\dot{V}_{O_2}$ increasing significantly during hypoxic exposure in LAR bar-headed geese ($P<0.001$),

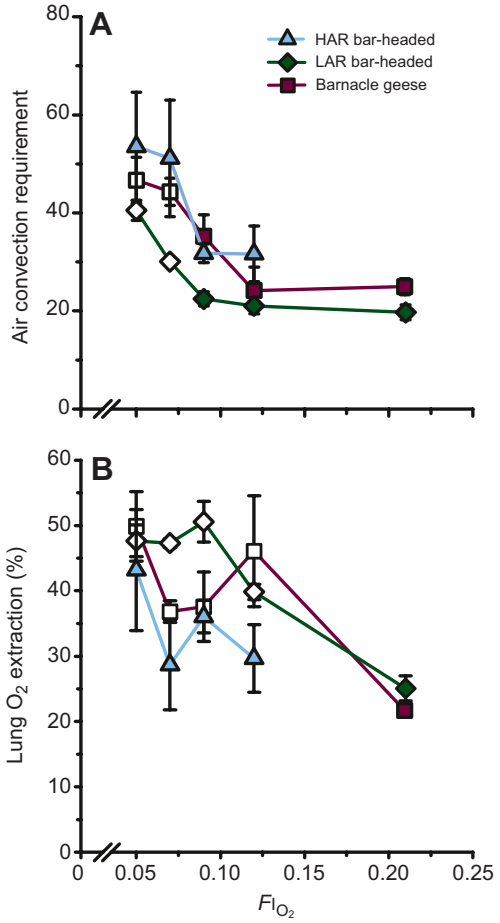

Fig. 2. Air convection requirement and lung oxygen extraction during progressive hypoxia. (A) Air convection requirement plotted as a function of the fractional oxygen composition of inspired gas ($F_{I_{O_2}}$) in all groups. (B) Changes in lung oxygen extraction in all groups exposed to progressive hypoxia. Values are reported in STPD and represent means±s.e.m. (blue: HAR bar-headed geese, N=5; green: LAR bar-headed geese, N=5; purple: barnacle geese, N=7). Significant differences ($P<0.05$) in values from those during ambient exposure within a species are indicated by open symbols and determined by one-way repeated measured ANOVA.

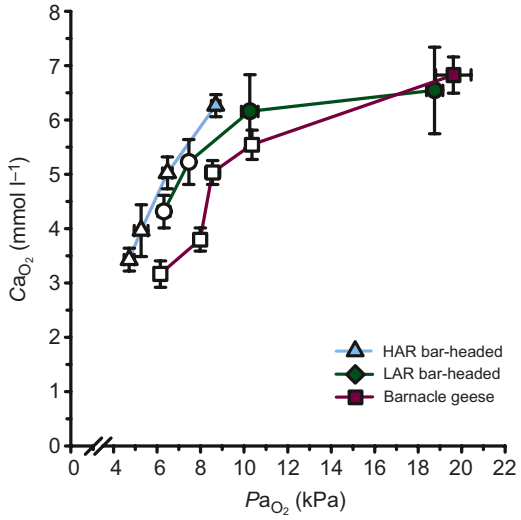

Fig. 3. Arterial oxygen content at a given level of arterial partial pressure differs between bar-headed geese and barnacle geese. The relationship between arterial oxygen content (Ca_{O_2}) and arterial partial pressure of oxygen (Pa_{O_2}) was such that at any given Pa_{O_2} bar-headed geese carried a greater content of O_2 in their arterial blood. Values are means±s.e.m. (blue: HAR bar-headed geese, N=5; green: LAR bar-headed geese, N=6; purple: barnacle geese, N=7). Significant differences ($P<0.05$) in Ca_{O_2} from values obtained at ambient exposures within a species are indicated by open symbols and determined by one-way repeated measured ANOVA.

Journal of Experimental Biology (2016) 219, 1974-1984 doi:10.1242/jeb.132431

Table 1. Changes in cardiovascular variables during hypoxic exposure in bar-headed geese and barnacle geese

$F_{I_{O_2}}$	Pa_{O_2} (kPa)	Pv_{O_2} (kPa)	Ca_{O_2} (mmol l^{-1})	Cv_{O_2} (mmol l^{-1})	pH_a	pH_v	MAP (kPa)	TPR (kPa min kg ml^{-1})	Blood convection requirement	Tissue O_2 delivery (ml min^{-1} kg^{-1})	Tissue O_2 extraction (%)
Bar-headed geese (0 m)											
(0.21)	18.8±0.4^{a}	7.6±0.8^{a}	6.5±0.8^{a}	4.0±0.4^{a}	7.57±0.02^{a}	7.54±0.03^{a}	26.4±0.8^{a}	0.08±0.01^{a}	24.4±6.8^{a}	52.8±11^{a}	36.8±6.8^{a}
0.12	10.3±0.4^{b}	–	6.2±0.7^{a}	2.9±0.6^{a}	7.66±0.02^{b}	–	25.1±0.9^{a}	0.11±0.04^{a}	16.2±3.8^{a}	33.0±9.2^{a}	53.3±8.2^{a}
0.09	7.5±0.1^{c}	–	5.2±0.4^{b}	3.1±0.7^{a}	7.72±0.03^{b}	–	24.5±0.8^{b}	0.11±0.02^{a}	22.0±5.1^{a}	28.4±6.5^{a}	44.2±10^{a}
0.07	6.3±0.3^{d}	4.7±0.3^{b}	4.3±0.3^{b}	2.9±0.4^{a}	7.76±0.03^{b}	7.69±0.03^{b}	23.4±0.8^{b}	0.05±0.00^{a}	32.0±3.6^{a}	49.3±0.6^{a}	34.1±4.5^{a}
Bar-headed geese (3200 m)											
0.12	8.7±0.2^{a}	–	6.3±0.2^{a}	3.7±0.4^{a}	7.72±0.01^{a}	–	20.8±1.2^{a}	0.09±0.01^{a}	20.7±3.5^{a}	24.8±5.8^{a}	38.7±7.0^{a}
0.09	6.5±0.2^{b}	–	5.0±0.3^{b}	3.9±0.2^{a}	7.78±0.02^{a}	–	21.2±0.8^{a}	0.06±0.01^{a}	41.2±5.7^{a}	44.1±3.4^{a}	23.1±2.5^{a}
0.07	5.3±0.3^{b}	–	4.0±0.5^{c}	2.7±0.6^{b}	7.79±0.04^{a}	–	19.5±0.6^{a}	0.04±0.01^{a}	35.4±6.7^{a}	50.6±8.6^{a}	32.3±7.7^{a}
0.05	4.7±0.2^{b}	3.4±0.4^{a}	3.4±0.2^{c}	1.8±0.4^{b}	7.72±0.06^{a}	7.65±0.06^{a}	19.5±0.9^{a}	0.05±0.01^{a}	29.3±4.4^{a}	33.1±4.5^{a}	48.4±7.9^{a}
Barnacle geese (0 m)											
(0.21)	19.6±0.8^{a}	–	6.8±0.3^{a}	5.0±0.4^{a}	7.62±0.01^{a}	–	21.3±0.9^{a}	0.10±0.01^{a}	25.8±3.0^{a}	36.4±5.3^{a}	27.5±3.4^{a}
0.12	10.4±0.2^{b}	–	5.5±0.3^{b}	3.5±0.2^{b}	7.69±0.02^{a}	–	19.0±0.9^{ab}	0.08±0.02^{a}	23.6±3.2^{a}	35.9±7.0^{a}	36.9±3.9^{a}
0.09	8.6±0.3^{c}	–	5.0±0.2^{b}	3.1±0.2^{bc}	7.77±0.03^{b}	–	17.7±1.1^{ab}	0.01±0.03^{a}	25.7±3.4^{a}	31.5±9.4^{a}	37.5±5.3^{a}
0.07	8.0±0.3^{c}	–	3.8±0.2^{c}	3.5±0.4^{bc}	7.71±0.05^{c}	–	17.4±1.4^{b}	0.05±0.01^{a}	36.1±7.7^{a}	32.9±7.5^{a}	37.8±10^{a}
0.05	6.2±0.2^{d}	–	3.2±0.2^{c}	1.9±0.1^{c}	7.8±0.04^{d}	–	19.8±1.9^{ab}	0.04±0.01^{a}	38.0±4.5^{a}	37.9±3.6^{a}	38.9±2.2^{a}

Changes in blood gases and cardiovascular variables with decreasing fractional inspired O_2 ($F_{I_{O_2}}$); Pa_{O_2}, arterial partial pressure of oxygen (kPa); Pv_{O_2}, venous partial pressure of oxygen (kPa); Ca_{O_2}, arterial content of oxygen (mmol l^{-1}); Cv_{O_2}, venous content of oxygen (mmol l^{-1}); pH_a, arterial pH; pH_v, venous pH; MAP, mean arterial pressure (kPa); TPR, total peripheral resistance (kPa); blood convection requirement; tissue O_2 delivery (ml min^{-1} kg^{-1}); and tissue O_2 extraction (%). Venous values were provided where available. ^{a,b,c,d}Significant differences (P<0.05) of a variable within species from values at ambient $F_{I_{O_2}}$ are indicated by different letters and determined by one-way repeated measured ANOVA.

almost significantly in HAR bar-headed geese (P=0.051) and remaining unchanged in barnacle geese (P=0.72). The $\dot{V}_{O_2}$ of the HAR bar-headed geese was significantly lower than that of the LAR bar-headed geese at every level except 0.07 $F_{I_{O_2}}$ (P=0.004).

Hypoxic ventilatory response

The hypoxic ventilatory responses of each study group and the differences present in the relative contributions of breathing frequency and tidal volume to $\dot{V}_R$ are depicted in a Hey plot

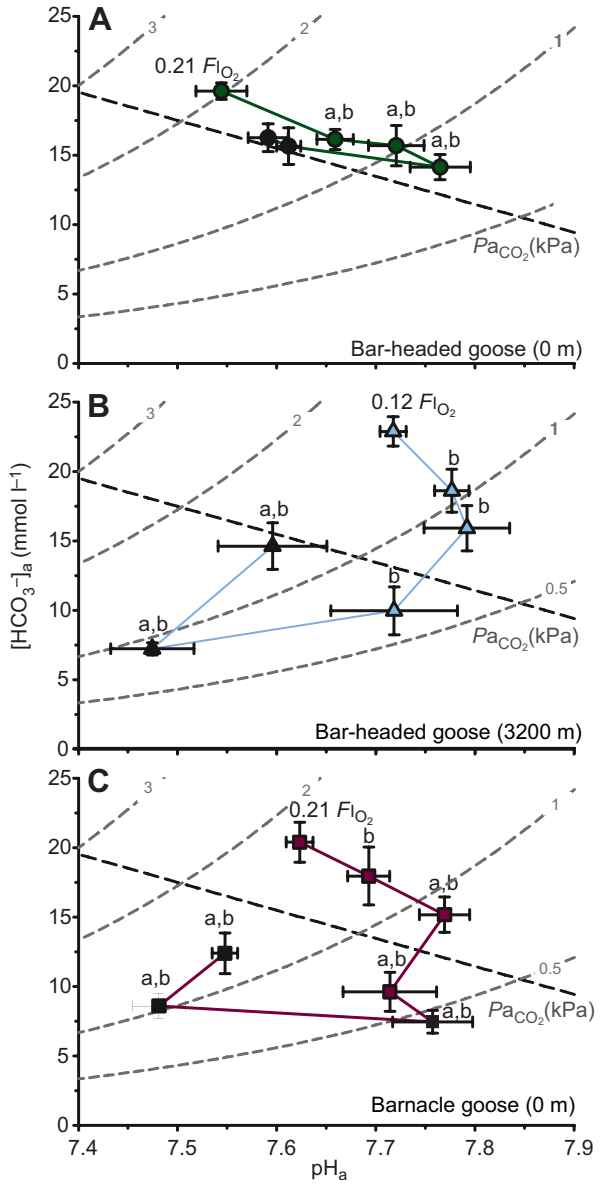

Fig. 4. Arterial acid-base status is altered during hypoxia exposure due to respiratory alkalosis and metabolic acidosis. These plots represent changes in arterial acid-base status (arterial pH, pH_a; arterial bicarbonate ion concentration, [HCO_3^-]) throughout the experimental exposures in (A) LAR bar-headed geese (N=6), (B) HAR bar-headed geese (N=5) and (C) barnacle geese (N=7). The first shaded point represents the starting ambient exposure and is marked with the corresponding $F_{I_{O_2}}$ (0.21 for low-altitude groups and 0.12 for the high-altitude group). Recovery in ambient $F_{I_{O_2}}$ after 5 and 25 min is represented by the black symbols. The dotted isopleths represent arterial partial pressure of CO_2 (Pa_{O_2}; kPa). Significant differences (P<0.05) in values from starting levels within a species are indicated by a for pH_a and b for [HCO_3^-] and determined by one-way repeated measured ANOVA.

Journal of Experimental Biology (2016) 219, 1974-1984 doi:10.1242/jeb.132431

(Fig. 1B), a graphical depiction of breathing patterns (tidal volume and breathing frequency) at different levels of $\dot{V}_R$ (Guz and Widdicombe, 1970). At a given level of $\dot{V}_R$, bar-headed geese breathed at a slower rate with significantly larger tidal volumes (Fig. 1B, Fig. S1A,B) than the barnacle geese, and this difference in pattern was sustained in hypoxia. The increase in $\dot{V}_R$ was greatest in the HAR bar-headed geese and was lowest for the barnacle geese (Fig. 1C). $\dot{V}_R$ of HAR bar-headed geese was higher than that of barnacle geese during every exposure (P<0.001). LAR bar-headed geese trended towards having a larger $\dot{V}_R$ than barnacle geese at 0.05 $F_{I_{O_2}}$, but this did not reach statistical significance (P=0.057). The air convection requirement – the ratio of $\dot{V}_R$ to $\dot{V}_{O_2}$ – increased in hypoxia in all groups, significantly so in both LAR groups (Fig. 2A). Lung O_2 extraction, which is the percentage of the inspired O_2 extracted from inspired gas, increased initially in both LAR groups between 0.21 and 0.12 $F_{I_{O_2}}$ (Fig. 2B), and then remained constant at 30–50% beyond 0.12 $F_{I_{O_2}}$. All differences in both the resting levels of $\dot{V}_R$ between HAR and LAR bar-headed geese disappeared when our data were expressed as STPD rather than BTPS (Fig. 1D).

Hypoxic cardiovascular response

Blood-O_2 carrying capacity and acid-base status

Hct and [Hb] were not significantly different among HAR bar-headed geese (Hct: 38.8±2.8%, [Hb]: 117.3±7.0 g l^{-1}), LAR bar-headed geese (Hct: 43.9±4.3%, [Hb]: 125.1±7.5 g l^{-1}), or LAR barnacle geese (Hct: 43.1±1.9%, [Hb]: 112.6±4.0 g l^{-1}). In all three groups of geese, Hct and [Hb] were unchanged during progressive hypobaric hypoxia.

Pa_{O_2} decreased with progressive decreases in $F_{I_{O_2}}$, and was lower (P<0.001) in the bar-headed geese than in barnacle geese at or below an $F_{I_{O_2}}$ of 0.07 (Fig. S2A). Ca_{O_2} was similar between the groups of geese and decreased with hypoxia (Fig. S2B). Plotting Ca_{O_2} as a function of Pa_{O_2} generated *in vivo* O_2 equilibrium curves (Fig. 3) that are representative of the arterial saturation given the prevailing acid-base conditions that accompanied hypobaric hypoxia (see Table 1). Differences in these O_2 equilibrium curves reflect the higher O_2 affinity of the bar-headed goose blood (Black and Tenney, 1980; Weber et al., 1993) compared with that of barnacle geese and differences in the pH_a at each $F_{I_{O_2}}$ (Fig. 4).

The starting pH_a of the HAR bar-headed geese was higher than that of the LAR bar-headed geese (P<0.001) and was accompanied by higher starting [HCO_3^-] (P=0.009) (Fig. 4A,B). Pa_{CO_2} decreased (P<0.001) and pH_a increased (P<0.001) in all three groups of geese during progressive hypoxia (Fig. 4, Table 1). Both bar-headed goose study groups experienced a respiratory alkalosis during hypoxic exposure down to 0.07 $F_{I_{O_2}}$ (Fig. 4A,B). At that point, LAR bar-headed geese were recovered to normoxia (Fig. 4A), whereas HAR bar-headed geese were further exposed to 0.05 $F_{I_{O_2}}$ (Fig. 4B). Between 0.07 and 0.05 $F_{I_{O_2}}$ (Fig. 4B), the pH_a of HAR bar-headed geese remained unchanged, but $[HCO_3^-]_a$ decreased significantly (Fig. 4B), indicative of a metabolic acidosis. This also occurred in the barnacle geese, but the metabolic acidosis was triggered at a less extreme level of hypoxia (0.07 $F_{I_{O_2}}$) (Fig. 4C). Intriguingly, only during ambient recovery did pH_a fall significantly in any group. LAR bar-headed geese recovered their pH_a within 5 min of normoxia after being exposed to 0.07 $F_{I_{O_2}}$. The HAR bar-headed geese and barnacle

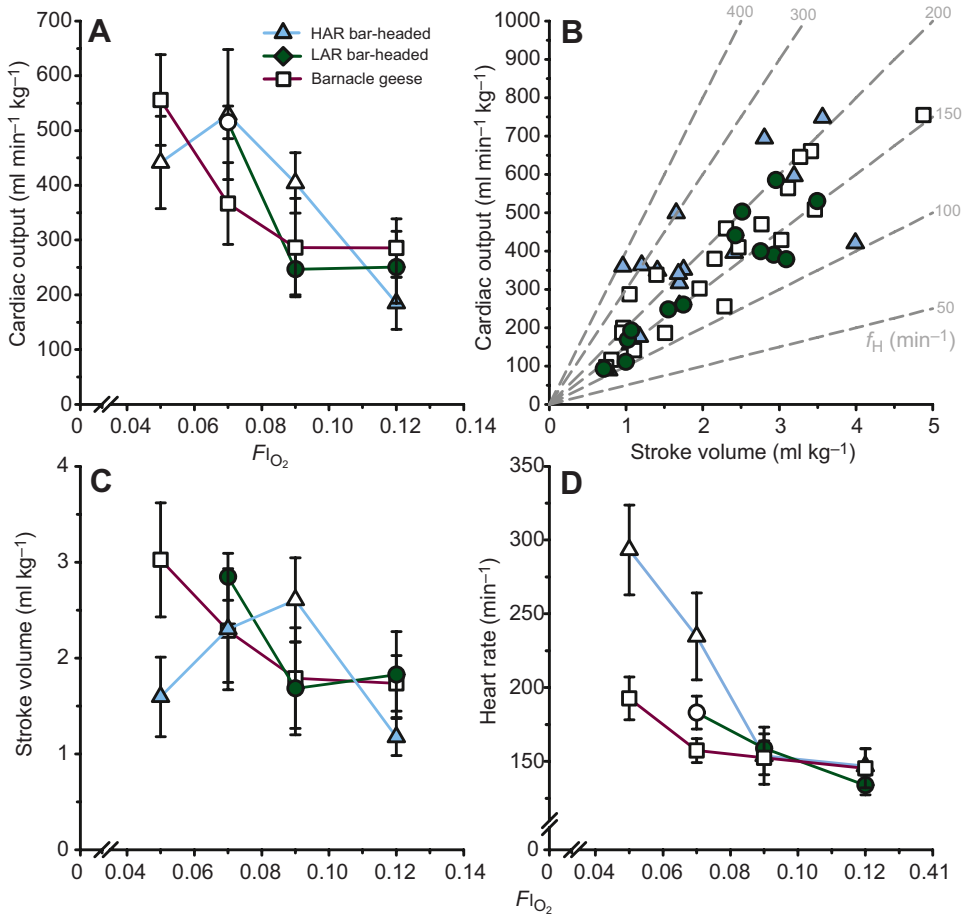

Fig. 5. Changes in cardiac output and its contributing components as a function of $F_{I_{O_2}}$. (A) Cardiac output plotted as a function of fractional oxygen composition of inspired gas ($F_{I_{O_2}}$). (B) The hypoxic cardiovascular response of all groups is characterized generally by large increases in stroke volume with modest increases in heart rate. All points represent individual data. Dashed lines represent heart rate (f_H) isopleths. (C) Stroke volume and (D) heart rate plotted as a function of $F_{I_{O_2}}$. Values are means± s.e.m. (blue: HAR bar-headed geese, N=5; green: LAR bar-headed geese, N=6; purple: barnacle geese, N=7). Significant differences (P<0.05) from values obtained with ambient exposure within a species are indicated by open symbols and determined by one-way repeated measured ANOVA.

Journal of Experimental Biology (2016) 219, 1974-1984 doi:10.1242/jeb.132431

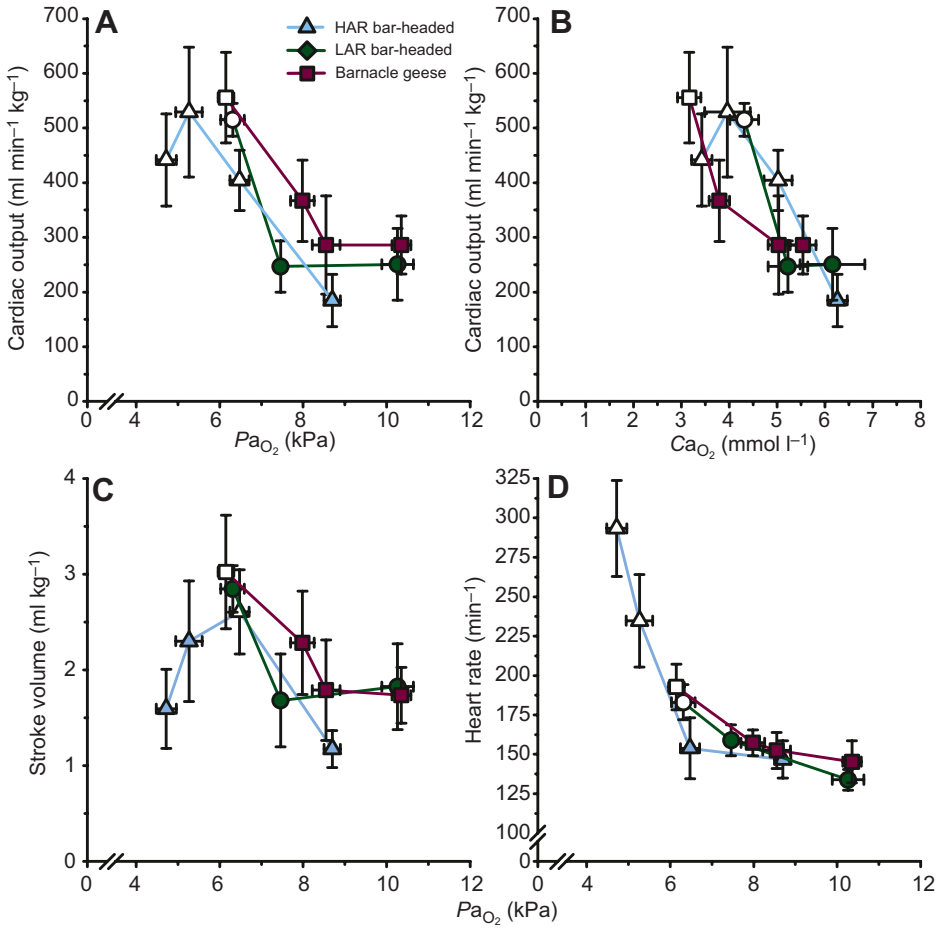

Fig. 6. Changes in cardiac output and its contributing components as a function of Pa_{O_2} or Ca_{O_2}. (A) Cardiac output plotted as a function of arterial partial pressure of oxygen (Pa_{O_2}). (B) Cardiac output plotted as a function of arterial oxygen content (Ca_{O_2}). (C) Stroke volume and (D) heart rate plotted as a function of Pa_{O_2}. All variables increased initially with decreasing at a Ca_{O_2} ~6 kPa. Values are means±s.e.m. (blue: HAR bar-headed geese, *N*=5; green: LAR bar-headed geese, *N*=6; purple: barnacle geese, *N*=7). Significant differences ($P<0.05$) from values obtained with ambient exposure within a species are indicated by open symbols and determined by one-way repeated measured ANOVA.

geese that were exposed to 0.05 $F_{I_{O_2}}$ both had a persistent acidosis and low $[HCO_3^-]_a$ even after 25 min of recovery (Fig. 4B,C).

Cardiac output

All three groups of geese increased $\dot{Q}$ by 2- to 3-fold, yielding similar maximum values during progressive hypoxia (Fig. 5A). The increase in $\dot{Q}$ became significant at a different $F_{I_{O_2}}$ between the groups: 0.09 in HAR bar-headed geese, 0.07 in LAR bar-headed geese and 0.05 in barnacle geese (Fig. 5A). The relative contributions of increases in heart rate and stroke volume to $\dot{Q}$ are depicted in a cardiac equivalent of a Hey plot for individuals at all exposures (Fig. 5B). Increases in stroke volume accounted for most of the increase in $\dot{Q}$ in barnacle geese and LAR bar-headed geese (Fig. 5B,C) - their heart rates increased only modestly (Fig. 5D) whereas stroke volume roughly doubled (Fig. 5C). This was also the case initially in the HAR bar-headed geese; however, at 0.07 $F_{I_{O_2}}$ heart rate increased substantially, with an associated decrease in stroke volume. The net overall result, however, was a trend for $\dot{Q}$ to increase earlier and more rapidly in bar-headed geese than barnacle geese, and more so in the HAR bar-headed geese than in the LAR bar-headed geese (Fig. 5A).

While the increase in $\dot{Q}$ of HAR bar-headed geese was triggered at a higher $F_{I_{O_2}}$, it is evident from Fig. 6A, which plots the changes in cardiac variables as a function of Pa_{O_2}, that this response was associated with this group of geese having a lower Pa_{O_2} at any given $F_{I_{O_2}}$ during hypoxia (Fig. S2A). Significant increases in $\dot{Q}$ occurred at the same Pa_{O_2} in all three groups of geese (~6 kPa). That this reflects the differences in the Hb–O_2 equilibrium curves is clear from the extent of the overlap when $\dot{Q}$ is plotted as a function of Ca_{O_2} (Fig. 6B). Similarly, significant changes in the contributions of stroke volume and heart rate to $\dot{Q}$ occurred at ≤6 kPa (Fig. 6C,D).

Tissue O_2 delivery and extraction

Neither blood convection requirement (the quotient of $\dot{Q}$ and $\dot{V}_{O_2}$) nor tissue O_2 delivery (the product of $\dot{Q}$ and Ca_{O_2}) changed significantly in any of the three groups of geese during progressive hypoxia (Table 1). The percentage of the O_2 extracted from arterial blood fluctuated between 30 and 50% and also did not change significantly either during progressive hypoxia or between any of the three groups of geese (Table 1).

Blood pressure and total peripheral resistance

While $\dot{Q}$ increased during hypoxic exposure, mean arterial pressure was generally maintained, decreasing minimally at 0.07 $F_{I_{O_2}}$ in the two LAR groups of geese (Table 1).

DISCUSSION

In this study we comprehensively compared the metabolic, ventilatory and cardiovascular responses of HAR bar-headed geese with those of LAR bar-headed geese. In addition, we compared these responses to those of barnacle geese, a member of a closely related genus, to provide further insight into responses unique to bar-headed geese. We found that HAR bar-headed geese exhibited a reduced $\dot{V}_{O_2}$ compared with LAR bar-headed geese. When exposed to progressive hypoxia, HAR bar-headed geese

Journal of Experimental Biology

Journal of Experimental Biology (2016) 219, 1974-1984 doi:10.1242/jeb.132431

exhibited a modestly increased HVR and initiated cardiac responses earlier than LAR bar-headed geese, supporting our initial hypothesis. However, the magnitude of these differences was not as large as those described for bar-headed geese during short-term acclimation to simulated high altitude (Black and Tenney, 1980). Explanations for these differences are discussed below.

While it cannot be determined with absolute certainty the extent to which the differences present between the HAR and LAR bar-headed geese can be attributed exclusively to differences in barometric pressure during rearing, many potentially confounding variables were controlled across study groups. All groups of birds were held in outdoor pens with access to indoor shelter during the winter. All groups were healthy, fed similar diets and had been housed for at least a year without flying. While body mass and body composition have been shown to vary seasonally in barnacle geese (Portugal et al., 2007), the relationship between heart rate and $\dot{V}_{O_2}$, when normalized for body mass, was unaffected in five of the six seasonal sampling periods and was also unaltered by molt (Portugal et al., 2009). Furthermore, many of the variables measured in this study have been previously measured on LAR bar-headed geese and barnacle geese, allowing us to compare results. Resting values for $\dot{V}_{O_2}$ and $\dot{V}_R$ in our LAR bar-headed geese in normoxia were comparable to those previously described in the literature (Black and Tenney, 1980; Fedde et al., 1989; Hawkes et al., 2014; Scott and Milsom, 2007). Our values for Ca_{O_2}, Cv_{O_2}, Hct and [Hb] also fell within the range of values previously reported in the literature (Black and Tenney, 1980; Fedde et al., 1989; Hawkes et al., 2014; Scott and Milsom, 2007). Literature values for $\dot{Q}$, stroke volume and heart rate of LAR bar-headed geese in normoxia vary widely. Fedde et al. (1989) reported a high heart rate and low stroke volume, while Hawkes et al. (2014) reported a low heart rate and high stroke volume. Our values fall midway between the two.

$\dot{V}_{O_2}$ was significantly lower in the HAR bar-headed geese compared with LAR bar-headed geese, suggesting that rearing at altitude leads to a reduction in metabolism and in the demand for O_2. All groups maintained or increased $\dot{V}_{O_2}$ when exposed to hypoxia (Fig. 1A). The small increases seen in $\dot{V}_{O_2}$ in all groups may reflect an increased cost of ventilation and associated events. The net response suggests that the cardiorespiratory adjustments were sufficient to match O_2 supply to O_2 demand at all but the most severe levels of hypoxia.

There was evidence of a metabolic acidosis, indicative of recruitment of anaerobic metabolism, in barnacle geese at 0.07 $F_{I_{O_2}}$ and in HAR bar-headed geese at 0.05 $F_{I_{O_2}}$. On return to control conditions, the HAR bar-headed geese recovered to control acid-base status faster (within 25 min) than the barnacle geese. Both groups exposed to 0.05 $F_{I_{O_2}}$ experienced a significant decrease in pH_a upon recovery to normoxia (Fig. 4B,C). This may reflect the sequestering of lactate and H^+ during hypoxia that was rapidly released into the blood upon return to resting conditions. Because LAR bar-headed geese were recovered to ambient conditions after breathing 0.07 $F_{I_{O_2}}$ rather than 0.05 $F_{I_{O_2}}$, we cannot ascertain whether the ability of bar-headed geese to avoid metabolic acidosis until more severe levels of hypobaric hypoxia is an adaptation or a consequence of high-altitude rearing. Nevertheless, the ability to recover quickly from severe hypoxia would be an asset during the high-altitude migration of bar-headed geese.

The magnitude by which $\dot{V}_R$ increased during progressive hypoxia in the LAR bar-headed geese and barnacle geese of our study was within the range reported in previous studies (Black and Tenney, 1980; Fedde et al., 1989; Hawkes et al., 2014; Scott and Milsom, 2007). In addition, as previously reported, bar-headed geese exhibited a higher overall tidal volume and lower breathing frequency at any given $\dot{V}_R$ than barnacle geese – a pattern hypothesized to be a more effective breathing pattern that reduces effective dead space ventilation (Scott and Milsom, 2007). The demonstration that this breathing pattern was common to LAR and HAR bar-headed geese supports the suggestion that this is an adaptation specific to bar-headed geese. Furthermore, we found that the increase in $\dot{V}_R$ in hypoxia was greatest in magnitude in the HAR bar-headed geese and lowest in the barnacle geese ($P<0.001$; Fig. 1C). These findings support our hypothesis and are consistent with previous findings of a greater increase in $\dot{V}_R$ in bar-headed geese following short-term acclimation to simulated altitude (Black and Tenney, 1980).

Differences in both the resting levels of ventilation and the HVR disappeared when our data were expressed as STPD rather than BTPS (Fig. 1D, Fig. S3B). Expressing volume as a function of STPD reveals the molar amount of air (and thus O_2) moved. In this instance, STPD values are not significantly different for either LAR or HAR bar-headed geese, indicating that the differences in ventilation reported in BTPS were due to the thinner air.

Expressing volumes as a function of BTPS, however, is standard for showing how much gas an animal ventilates. Black and Tenney found that the resting ventilation in bar-headed geese acclimated under hypobaric conditions (equivalent to 5640 m) for 4 weeks was approximately double that of sea level-acclimated birds when measured at similar levels of Pa_{O_2} under normobaric conditions. In the present study, while the level of $\dot{V}_R$ at an inspired partial pressure of O_2 of 12 kPa ($F_{I_{O_2}}$=0.21 at 3200 m; sea level equivalent $F_{I_{O_2}}$=0.12) was roughly 30% higher in the HAR bar-headed geese relative to the LAR bar-headed geese, this difference was not significant. This suggests that despite apparent similarities, the changes seen following short-term acclimation (Black and Tenney, 1980) are more akin to ventilatory acclimatization to hypoxia, while those seen in the HAR bar-headed geese appear to reflect hypoxic desensitization (Powell et al., 1998).

Ventilatory acclimatization to hypoxia is defined as the further increase in ventilation, compared with the rapid initial response, which occurs over hours to days of acclimatization (Powell et al., 1998). This secondary increase has been ascribed to plasticity in O_2 sensing by the carotid body chemoreceptors and in central integration of chemoreceptor input (Powell, 2007). Over many months at high altitude, however, this hypoxic ventilatory response can be gradually attenuated by hypoxic desensitization (Brutsaert, 2007; Powell et al., 1998). While the increases in breathing during ventilatory acclimatization to hypoxia improve O_2 uptake, hypoxic desensitization could be representative of longer-term high-altitude exposure and the ability to effectively transport O_2 without magnified convective transport. This would help reduce respiratory water loss, and reduce the metabolic cost of breathing (Powell, 2007; Storz et al., 2010). Despite the apparent hypoxic desensitization, $\dot{V}_R$ remained elevated relative to $\dot{V}_{O_2}$ in the HAR bar-headed geese, indicative of a reduction in lung O_2 extraction (Fig. 2).

Differences between species in blood-O_2 carrying capacity were driven primarily by differences in intrinsic O_2 affinity (i.e. P_{50} of Hb) and *in vivo* blood O_2 affinity (i.e. blood O_2 affinity subject to *in vivo* changes in temperature, pH and allosteric modulators). There were no inter- or intraspecies differences in [Hb] or Hct either in normoxia or with progressive hypoxia. The greater O_2 affinity of the HbA isoform of bar-headed geese is well documented (Weber et al., 1993), although the properties of the HbD isoform have yet to be studied. Neither Hct nor [Hb] in bar-headed geese were affected by

Journal of Experimental Biology (2016) 219, 1974-1984 doi:10.1242/jeb.132431

rearing altitude, a finding also reported after short-term (4 weeks) acclimation of bar-headed geese to simulated altitude (5640 m) (Black and Tenney, 1980). This is also similar to patterns described in high-altitude-acclimatized Tibetan humans (Simonson et al., 2015) and deer mice (Lui et al., 2015). As a result of the differences in intrinsic and *in vivo* blood O_2 affinity, however, at any given Pa_{O_2} bar-headed goose blood will be more saturated than that of barnacle geese (Fig. 3). Furthermore, the data suggest that at any given Pa_{O_2}, the blood of the HAR bar-headed geese would be slightly more saturated than that of the LAR bar-headed geese (Fig. 3). These small differences are most likely explained by the higher levels of $[HCO_3^-]_a$ (P=0.009) at 0.12 $F_{I_{O_2}}$ and pH_a (P<0.001) at all levels of hypoxia in the high-altitude study group. Such an alkalosis would left-shift the O_2 equilibrium curve and enhance O_2 loading at the lung, which are possibly another features of high-altitude rearing. A respiratory alkalosis occurred in all groups during progressive hypoxic exposure due to heavy ventilation, further enhancing blood-O_2 carrying capacity (Fig. 4).

Based on the *in vivo* O_2 equilibrium curves, the blood P_{50} of the two groups of bar-headed geese are unlikely to be appreciably different. While birds have organic phosphates (inositol pentophosphate) for altering the O_2 affinity of Hb, there is little evidence of an IPP-induced change in P_{50} with high-altitude exposure (Weber, 2007). This suggests that isoHb switching did not occur in response to environmental hypoxia. Although large reversible changes in blood P_{50} could be achieved by altering the expression levels of HbA and HbD, our data suggest that bar-headed geese do not do this. Similar results have been reported for high- versus low-altitude hummingbirds (Projecto-Garcia et al., 2013), sparrows (Cheviron et al., 2014), house wrens (Galen et al., 2015) and waterfowl (Natarajan et al., 2015).

All groups in the present study increased $\dot{Q}$ 2.0- to 2.5-fold during severe hypoxia. Previous reports of the magnitude and direction of change in $\dot{Q}$ during severe hypoxia in bar-headed geese vary widely. Hawkes et al. (2014) reported that, in bar-headed geese breathing 0.07 $F_{I_{O_2}}$, $\dot{Q}$ decreased by ∼20%, whereas Fedde et al. (1989) and Black and Tenney (1980) reported no change in $\dot{Q}$ at this level of hypoxia. However, when Black and Tenney exposed their birds to a further reduction in O_2 to ∼0.05 $F_{I_{O_2}}$, corresponding to a Pa_{O_2} of ∼3.5 kPa, $\dot{Q}$ increased by a remarkable 7-fold (Black and Tenney, 1980). These differences most likely reflect the steepness of the exponential cardiovascular response curve beyond the inflection point and small differences in Pa_{O_2}. Analysis of the data based on $F_{I_{O_2}}$ suggests that increases in $\dot{Q}$ in the present study were initiated first in the HAR bar-headed geese (0.09 $F_{I_{O_2}}$), next in the LAR bar-headed geese (0.07 $F_{I_{O_2}}$) and last in the barnacle geese (0.05 $F_{I_{O_2}}$) (Fig. 5A). When expressed as a function of Pa_{O_2} (or Ca_{O_2}), however, all groups produced significant increases in $\dot{Q}$ at a similar Pa_{O_2} of ∼6 kPa (Fig. 6A, Fig. S3C), indicating that the differences in which $\dot{Q}$ increases are initiated when plotted as a function of $F_{I_{O_2}}$ reflect differences in the blood O_2 affinity. Black and Tenney made a similar observation. They too noted that the differences they saw in the changes in $\dot{Q}$ during progressive hypoxia following short-term acclimation in bar-headed geese could be accounted for by differences in Ca_{O_2} (Black and Tenney, 1980). Unlike Black and Tenney, however, we did not see an increase in the overall magnitude of $\dot{Q}$ at a given Pa_{O_2} with lifelong exposure to high altitude. Under resting conditions, $\dot{Q}$ does not differ in high- versus low-altitude native or domestic mammals either. Total blood flow has been found to be unaltered or slightly reduced in humans, alpaca, llama, rats and wild mice living at altitude (Banchero et al., 1971; Klausen, 1966; Monge et al., 1955; Sillau et al., 1976; Turek et al., 1973). Thus, the differences present between the study of short-term acclimatization by Black and Tenney (1980) and of high-altitude rearing in our study not only suggest that both acclimatization to hypoxia and hypoxic desensitization occur with ventilatory responses, but also that similar acclimatization and desensitization to hypoxia occur with regard to blood flow in bar-headed geese, and may act to reduce the costs of convective transport of blood as they do for respiratory gases.

Previous studies on geese reported changes in heart rate as the primary contributor to changes in $\dot{Q}$, with stroke volume remaining largely unchanged (Faraci, 1986; Fedde et al., 1989; Smith et al., 2000). In the present study, this was true of the low-altitude groups down only to an $F_{I_{O_2}}$ of 0.12. Below this, all groups increased $\dot{Q}$ during progressive hypoxia down to a Pa_{O_2} ∼6 kPa (Fig. S3C) more by increasing stroke volume than heart rate (Fig. 5C and Fig. 6C). HAR bar-headed geese were the only group in which Pa_{O_2} fell <6 kPa, at which point heart rate increased substantially (Fig. 6D) associated with a decrease in stroke volume (Fig. 6C). The large increases in stroke volume seen in the present study could have been mediated either by extrinsic factors (circulating hormones or neurotransmitters) or intrinsic factors [cardiac muscle fiber contractile properties associated with the Frank–Starling response – the relationship between cardiac contractility and venous return (Smith et al., 2000)]. As outlined in Shiels and White (2008), limited information exists on the Frank–Starling response in avian cardiomyocytes, although it is known to facilitate large increases in stroke volume during hypoxia in fish (Farrell, 1991; Shiels and White, 2008). Further studies are required to determine the underlying mechanisms of this response.

Conclusions

The primary differences present between the HAR and LAR bar-headed geese were ventilatory and metabolic in nature. But, at this point, we cannot discern the differential effects of phenotypic plasticity (i.e. acclimatization) from developmental plasticity on the physiology of this species. The reduction in resting $\dot{V}_{O_2}$ was one of the most significant differences observed in the HAR bar-headed geese. We also observed an increase in resting $\dot{V}_R$ and in the HVR that could be explained by the differences in barometric pressure at which the measurements were made. Even taking this into account, however, HAR bar-headed geese still exhibited a large air convection requirement (ratio of $\dot{V}_R$ to $\dot{V}_{O_2}$), compensating for a reduction in lung O_2 extraction (Fig. S3A). This may help to maintain blood acid–base balance at the expense of O_2 uptake.

All geese increased $\dot{Q}$ by ∼2-fold to a similar overall magnitude, but $\dot{Q}$ increased earlier and more rapidly in bar-headed geese than barnacle geese as environmental O_2 fell, and more so in the HAR bar-headed geese than in LAR bar-headed geese. However, this could be explained by the differences in *in vivo* blood O_2 affinity. All groups increased perfusion at a similar Pa_{O_2} during hypoxic exposure. An unexpected finding was the prominent role of increases in stroke volume in increasing $\dot{Q}$ in all groups, including the barnacle geese. Further studies are required to determine the underlying mechanisms of the differences reported here between HAR bar-headed geese and LAR bar-headed geese, and the extent to which these differences may also facilitate high-altitude flight.

Acknowledgements

We thank J. Meir, M. Pamenter, M. Qu, E. J. Ross, J. York, and J. You for their assistance in performing these experiments. We also thank Dr G. R. Scott for his insights concerning these data. We express our gratitude to Dr Yang Zhong of Fudan University for assisting us in logistical arrangements in the field, as well as to the Lake Qinghai Wildlife Conservation Office for their field and lab assistance. We thank

the staff at the Centre for Comparative Medicine at the University of British Columbia for their care of the low-altitude experimental animals, as well as B. Gillespie and V. Grant at the University of British Columbia for building the experimental apparatus. Finally we would like to thank the anonymous reviewers whose comments greatly helped shape this manuscript.

Competing interests
The authors declare no competing or financial interests.

Author contributions
S.L.L. designed the study, carried out all lab work and field data collection, completed all data analysis, and drafted the manuscript. B.C. carried out all surgical procedures and helped collect field data. A.P.F. participated in study design and helped to draft the manuscript. Y.W. participated in study design, helped to carry out and coordinate field work. W.K.M. participated in study design, helped to carry out and coordinate field work, and helped to draft the manuscript. All authors edited the manuscript and gave final approval for publication.

Funding
Our research was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grants to W.K.M. and A.P.F. A.P.F. also holds a Canada Research Chair. S.L.L. was supported by an NSERC Vanier Canada Graduate Scholarship and an Izaak Walton Killam Memorial Doctoral Scholarship awarded by the University of British Columbia. Y.W. was supported by an NSERC Discovery Grant, the Canadian Innovation Foundation and research awards from the National Natural Science Foundation of China and Ministry of Education.

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.132431/-/DC1

References
Banchero, N., Grover, R. F. and Will, J. A. (1971). Oxygen transport in the llama (*Lama glama*). *Respir. Physiol.* **13**, 102-115.
Bavis, R. W. (2005). Developmental plasticity of the hypoxic ventilatory response after perinatal hyperoxia and hypoxia. *Respir. Physiol. Neurobiol.* **149**, 287-299.
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. and Tenney, S. M. (1980). Oxygen transport during progressive hypoxia in high-altitude and sea-level waterfowl. *Respir. Physiol.* **39**, 217-239.
Brutsaert, T. D. (2007). Population genetic aspects and phenotypic plasticity of ventilatory responses in high altitude natives. *Respir. Physiol. Neurobiol.* **158**, 151-160.
Carey, C., Thompson, E. and Vleck, C. (1982). Avian reproduction over an altitudinal gradient: incubation period, hatchling mass, and embryonic oxygen consumption. *Auk* **99**, 710-718.
Cheviron, Z. A., Bachman, G. C., Connaty, A. D., McClelland, G. B. and Storz, J. F. (2012). Regulatory changes contribute to the adaptive enhancement of thermogenic capacity in high-altitude deer mice. *Proc. Natl. Acad. Sci. USA* **109**, 8635-8640.
Cheviron, Z. A., Natarajan, C., Projecto-Garcia, J., Eddy, D. K., Jones, J., Carling, M. D., Witt, C. C., Moriyama, H., Weber, R. E., Fago, A. et al. (2014). Integrating evolutionary and functional tests of adaptive hypotheses: a case study of altitudinal differentiation in hemoglobin function in an Andean sparrow, *Zonotrichia capensis*. *Mol. Biol. Evol.* **31**, 2948-2962.
Dejours, P. (1975). Appendix. In *Principles of Comparative Respiratory Physiology*, pp. 215-223. New York: Elsevier.
Faraci, F. M. (1986). Circulation during hypoxia in birds. *Comp. Biochem. Physiol. A Physiol.* **85**, 613-620.
Farrell, A. (1991). From hagfish to tuna: a perspective on cardiac function in fish. *Physiol. Zool.* **64**, 1137-1164.
Fedde, M. R., Orr J.A., Shams H. and Scheid P. (1989). Cardiopulmonary function in exercising bar-headed geese during normoxia and hypoxia. *Respir. Physiol.* **77**, 239-252.
Ferner, K. and Mortola, J. P. (2009). Ventilatory response to hypoxia in chicken hatchlings: a developmental window of sensitivity to embryonic hypoxia. *Respir. Physiol. Neurobiol.* **165**, 49-53.
Galen, S. C., Natarajan, C., Moriyama, H., Weber, R. E., Fago, A., Benham, P. M., Chavez, A. N., Cheviron, Z. A., Storz, J. F. and Witt, C. C. (2015). Contribution of a mutational hot spot to hemoglobin adaptation in high-altitude Andean house wrens. *Proc. Natl. Acad. Sci. USA* **112**, 13958-13963.
Guz, A. and Widdicombe, J. G. (1970). Pattern of breathing during hypercapnia before and after vagal blockade in man. In *Breathing: Herin-Bruer Centenary Symposium* (ed. R. Porter), pp. 41-52. London: J. & A. Churchill.
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*). *Proc. Natl. Acad. Sci. USA* **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.
Ivy, C. M. and Scott, G. R. (2015). Control of breathing and the circulation in high-altitude mammals and birds. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **186**, 66-74.
Klausen, K. (1966). Cardiac output in man in rest and work during and after acclimatization to 3,800 m. *J. Appl. Physiol.* **21**, 609-616.
León-Velarde, F. and Monge-C, C. (2004). Avian embryos in hypoxic environments. *Respir. Physiol. Biol.* **141**, 331-343.
Lui, M. A., Mahalingam, S., Patel, P., Connaty, A. D., Ivy, C. M., Cheviron, Z. A., Storz, J. F., McClelland, G. B. and Scott, G. R. (2015). High-altitude ancestry and hypoxia acclimation have distinct effects on exercise capacity and muscle phenotype in deer mice. *Am. J. Physiol.* **308**, R779-R791.
McClelland, G. B., Hochachka, P. W. and Weber, J.-M. (1998). Carbohydrate utilization during exercise after high-altitude acclimation: a new perspective. *Proc. Natl. Acad. Sci. USA* **95**, 10288-10293.
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.
Monge, C., Cazorla, A., Whittembury, G., Sakata, Y. and Rizo-Patron, C. (1955). A description of the circulatory dynamics in the heart and lungs of people at sea level and at high altitude by means of the dye dilution technique. *Acta. Physiol. Lat. Am.* **5**, 198-210.
Mortola, J. P. (2011). Metabolic and ventilatory sensitivity to hypoxia in avian embryos. *Respir. Physiol. Neurobiol.* **178**, 174-180.
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.
Portugal, S. J., Green, J. A. and Butler, P. J. (2007). Annual changes in body mass and resting metabolism in captive barnacle geese (*Branta leucopsis*): the importance of wing moult. *J. Exp. Biol.* **210**, 1391-1397.
Portugal, S. J., Green, J. A., Cassey, P., Frappell, P. B. and Butler, P. J. (2009). Predicting the rate of oxygen consumption from heart rate in barnacle geese Branta leucopsis: effects of captivity and annual changes in body condition. *J. Exp. Biol.* **212**, 2941-2948.
Powell, F. L. (2007). The influence of chronic hypoxia upon chemoreception. *Respir. Physiol. Neurobiol.* **157**, 154-161.
Powell, F., Milsom, W. K. and Mitchell, G. S. (1998). Time domains of the hypoxic ventilatory response. *Respir. Physiol.* **112**, 123-134.
Projecto-Garcia, J., Natarajan, C., Moriyama, H., Weber, R. E., Fago, A., Cheviron, Z. A., Dudley, R., McGuire, J. A., Witt, C. C. and Storz, J. F. (2013). Repeated elevational transitions in hemoglobin function during the evolution of Andean hummingbirds. *Proc. Natl. Acad. Sci. USA* **110**, 20669-20674.
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. (2006). Flying high: a theoretical analysis of the factors limiting exercise performance in birds at altitude. *Respir. Physiol. Neurobiol.* **154**, 284-301.
Scott, G. R. and Milsom, W. K. (2007). Control of breathing and adaptation to high altitude in the bar-headed goose. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **293**, R379-R391.
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.
Shiels, H. A. and White, E. (2008). The Frank-Starling mechanism in vertebrate cardiac myocytes. *J. Exp. Biol.* **211**, 2005-2013.
Sillau, A. H., Cueva, S., Valenzuela, A. and Candela, E. (1976). O2 transport in the alpaca (*Lama pacos*) at sea level and at 3,300 m. *Respir. Physiol.* **27**, 147-155.
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: Sturkie's Avian Physiology.

Snyder, G. K., Black, C. P. and Birchard, G. F. (1982). Development and metabolism during hypoxia in embryos of high altitude *Anser indicus* versus sea level Branta canadensis geese. *Physiol. Zool.* **55**, 113-123.
Storz, J. F., Scott, G. R. and Cheviron, Z. A. (2010). Phenotypic plasticity and genetic adaptation to high-altitude hypoxia in vertebrates. *J. Exp. Biol.* **213**, 4125-4136.
Szdzuy, K. and Mortola, J. P. (2007). Ventilatory chemosensitivity of the 1-day-old chicken hatchling after embryonic hypoxia. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **293**, R1640-R1649.
Tucker, V. (1967). Method for oxygen content and dissociation curves on microliter blood samples. *J. Appl. Physiol.* **23**, 410-414.
Turek, Z., Grandtner, M., Ringnalda, B. E. M. and Kreuzer, F. (1973). Hypoxic pulmonary steady-state diffusing capacity for CO and cardiac output in rats born at a simulated altitude of 3500 m. *Pflugers Arch.* **340**, 11-18.
Weber, R. (2007). High-altitude adaptations in vertebrate hemoglobins. *Respir. Physiol. Neurobiol.* **158**, 132-142.
Weber, R., Jessen, T. and Malte, H. (1993). Mutant hemoglobins (alpha 119-Ala and beta 55-Ser): functions related to high-altitude respiration in geese. *J. Appl. Physiol.* **75**, 2646-2655.
Withers, P. C. (1977). Measurement of VO2, VCO2, and evaporative water loss with a flow-through mask. *J. Appl. Physiol.* **42**, 120-123.