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SHORT COMMUNICATION

High thermal sensitivity of blood enhances oxygen delivery in the high-flying bar-headed goose

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SUMMARY

The bar-headed goose (*Anser indicus*) crosses the Himalaya twice a year at altitudes where oxygen (O_2) levels are less than half those at sea level and temperatures are below –20°C. Although it has been known for over three decades that the major hemoglobin (Hb) component of bar-headed geese has an increased affinity for O_2, enhancing O_2 uptake, the effects of temperature and interactions between temperature and pH on bar-headed goose Hb–O_2 affinity have not previously been determined. An increase in breathing of the hypoxic and extremely cold air experienced by a bar-headed goose at altitude (due to the enhanced hypoxic ventilatory response in this species) could result in both reduced temperature and reduced levels of CO_2 at the blood–gas interface in the lungs, enhancing O_2 loading. In addition, given the strenuous nature of flapping flight, particularly in thin air, blood leaving the exercising muscle should be warm and acidotic, facilitating O_2 unloading. To explore the possibility that features of blood biochemistry in this species could further enhance O_2 delivery, we determined the P_{50} (the partial pressure of O_2 at which Hb is 50% saturated) of whole blood from bar-headed geese under conditions of varying temperature and [CO_2]. We found that blood–O_2 affinity was highly temperature sensitive in bar-headed geese compared with other birds and mammals. Based on our analysis, temperature and pH effects acting on blood–O_2 affinity (cold alkalotic lungs and warm acidotic muscle) could increase O_2 delivery by twofold during sustained flapping flight at high altitudes compared with what would be delivered by blood at constant temperature and pH.

Key words: blood–oxygen affinity, oxygen–hemoglobin equilibrium curve, temperature effect, CO_2 Bohr effect, *Anser indicus*, high altitude.

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INTRODUCTION

Bar-headed geese [*Anser indicus* (Latham 1790)] undertake an extraordinary high-altitude migration over the Himalaya, flying between their wintering grounds in southern Asia and their breeding grounds in the Central Asian Highlands twice a year. Flapping flight is sustained in these birds at altitudes above 7000 m (Hawkes et al., 2013; Hawkes et al., 2011), where oxygen (O_2) levels are less than half of those at sea level and temperatures are below −20°C. Over three decades ago, the primary hemoglobin component (HbA) of the bar-headed goose was shown to have a greater affinity for O_2 than most other birds (Black and Tenney, 1980; Petschow et al., 1977), enhancing O_2 uptake in these high-fliers. Bar-headed geese also have an enhanced hypoxic ventilatory response, increasing their breathing substantially more in response to hypoxia than do other birds (Scott and Milsom, 2007) and producing a severe respiratory alkalosis. Consequently, an increase in breathing of the hypoxic and extremely cold air experienced by a bar-headed goose at altitude could result in both reduced levels of CO_2 and reduced temperature at the blood–gas interface in the lungs. As increases in pH and reductions in temperature increase the affinity of hemoglobin (Hb) for O_2 (the Bohr and temperature effects), this should significantly enhance loading of O_2. In addition, given the strenuous nature of flapping flight, particularly in thin air (Hawkes et al., 2011), blood leaving the exercising muscle will be warm and acidotic, decreasing Hb–O_2 affinity and facilitating O_2 unloading.

Although temperature and pH modulate the binding properties of most Hbs, the extent to which they do so is species specific. Reductions in the thermal sensitivity of Hbs have been demonstrated in several vertebrate species, from active fishes (Rossi-Fanelli and Antonini, 1960) to ruminants (De Rosa et al., 2004), hummingbirds (Johansen et al., 1987) and even the woolly mammoth (Campbell et al., 2010). Though the specific mechanisms behind these reductions in the temperature dependence of O_2 binding affinity vary and indicate multiple evolutionary origins, their convergent physiological function protects O_2 delivery in these regionally heterothermic animals (Weber and Campbell, 2011). The conditions of high-altitude flight in the bar-headed goose, with a potentially cold and hypocapnic respiratory surface and warm, exercising muscles, however, pose an intriguing alternative scenario. Could temperature or pH effects be enhanced in species adapted to performance at high altitude? In order to explore additional features of blood biochemistry and assess the relative contribution of these factors to O_2 delivery in bar-headed geese, we determined the P_{50} [partial pressure of O_2 (P_{O_2}) at which Hb is 50% saturated] of whole blood from bar-headed geese under conditions of varying temperature and [CO_2]. We hypothesized that (1) bar-headed goose blood would have an increased temperature dependence of O_2 binding as compared with other birds, enhancing temperature effects in this species, and (2) the combined effects of temperature and pH on the blood–O_2 binding

properties of bar-headed geese could result in a significant increase in O_2 loading at the lungs and unloading at exercising muscle during high-altitude migration.

MATERIALS AND METHODS

Blood samples were drawn *via* standard venipuncture of the jugular vein of bar-headed geese (N=6, mass=2.39±0.32 kg) maintained in captivity at the University of British Columbia (UBC). All geese used for this study were hatched and raised at sea level and thus had no previous exposure to altitude or hypoxia. All procedures were approved by the UBC Animal Care Committee. Blood samples were placed on ice and processed immediately. P_{50} values of fresh whole blood were determined with the mixing technique using tonometered blood (Scheid and Meyer, 1978). All analyses were completed within 6 h of blood collection in order to minimize depletion of labile organic phosphates, such as inositol pentaphosphate, that would influence the dissociation curve, and to avoid prolonged cellular metabolism. The mixing technique consisted of two steps: (1) 1 ml of blood was placed in each tonometer and equilibrated for 35 min to achieve 0% O_2 saturation or 100% O_2 saturation at the appropriate %CO_2 and temperature [using custom-mixed 1%, 4% or 7% CO_2 with a balance of nitrogen for the 0% O_2-saturated blood and a balance of air for the 100% O_2-saturated blood (Praxair, Vancouver, BC, Canada)]; and (2) equal parts (0.4 ml each) of the 0% O_2-saturated blood and 100% O_2-saturated blood were mixed to achieve 50% O_2 saturation (S_{O2}). P_{O2} of the resulting mixture was measured using an i-STAT blood gas analyzer (37°C; Abaxis North America, Union City, CA, USA). Use of the i-STAT analyzer (CG4+ cartridge) also allowed measurement of pH and P_{CO2}. P_{50} values were determined under all combinations of the following conditions: temperature=37, 41 and 44°C, and %CO_2=1, 4 and 7% [P_{CO2}=7.6, 30.4 and 53.2 mmHg (1.01, 4.05 and 7.09 kPa), respectively]. These varying levels of CO_2 also allowed for calculation of the CO_2 Bohr effect. To minimize variability arising from cell metabolism, the order of temperature/CO_2 treatments for each animal was randomized, samples for each individual P_{50} data point were tonometered separately, and samples were left on ice until needed for tonometry. When temperature was not 37°C, P_{O2} was corrected using the encoded i-STAT temperature correction algorithms. These specific tonometry and mixing technique protocols have been validated in previous studies (Meir et al., 2009; Meir and Ponganis, 2009).

The temperature coefficient was determined as $\Delta\log P_{50}/\Delta T$, using measurements at the largest change in temperature (37 to 44°C) (data from all birds combined), and was converted to overall enthalpy of oxygenation, $\Delta H'$, using the van't Hoff equation [$\Delta H'=2.303R\Delta\log P_{50}/\Delta(1/T)$], where R is the gas constant (kJ mol^{-1}) and T is the absolute temperature (K). The CO_2 Bohr coefficient was determined as $\Delta\log P_{50}/\Delta$pH, using the measured P_{50} and pH values at 1% and 7% CO_2.

Additional blood samples were drawn for Hb (and hematocrit) analyses to allow for O_2 content calculation (N=16 geese). Hemoglobin concentration was determined with the cyanomethemoglobin technique. Hematocrit was determined by standard capillary tube centrifugation (14,000 r.p.m., 5 min).

Statistical significance was assumed at P<0.05 and the significance level is quoted in the text. Values are expressed as means ± s.d.

RESULTS

As one goose had unusually high lactate levels in the blood, results from this individual were not included in subsequent analysis. The P_{50} of whole blood from bar-headed geese was 31.2±2.5 mmHg (4.16±0.33 kPa; N=5) at 41°C and pH ~7.4 (4% inspired CO_2), similar to previously published values (Black and Tenney, 1980; Petschow et al., 1977). The CO_2 Bohr coefficient (over the full range studied) was −0.48±0.06 at 37°C, −0.42±0.02 at 41°C and −0.48±0.09 at 44°C (Fig. 1). These values were not significantly different (one-way ANOVA, F=1.25, P=0.32). The temperature coefficients (and corresponding $\Delta H'$) ranged from 0.032±0.005 (−60.2±9.9 kJ mol^{-1}) in blood equilibrated with 1% CO_2 to 0.028±0.005 (−52.8±9.0 kJ mol^{-1}) in blood equilibrated with 4% CO_2 and 0.024±0.003 (−45.7±5.7 kJ mol^{-1}) in blood equilibrated with 7% CO_2 (Fig. 1). Again, these values were not significantly different (one-way ANOVA, F=3.74, P=0.06). Thus the magnitude of the change in the P_{50} of the blood on warming from 37 to 44°C [11.2±2.2 mmHg (1.49±0.29 kPa) at 1% CO_2, 13.6±2.5 mmHg (1.81±0.33) at 4% CO_2 and 13.9±1.3 mmHg (1.85±0.17 kPa) at 7% CO_2] was independent of the level of CO_2 to which the blood was equilibrated. Hb concentration was 17.1±1.24 g dl^{-1} (2.59±0.19 mmol l^{-1}) and hematocrit 43.3±3.9% (N=16).

DISCUSSION

The temperature coefficient of whole blood from bar-headed geese (0.028) corresponds to a shift of the P_{50} of ~1.84 mmHg °C^{-1} and is: (1) ~24–43% greater than that of other birds examined to date (Danzer and Cohn, 1967; Johansen et al., 1987; Maginniss et al., 1997; Pinshow et al., 1985), (2) 18–50% greater than that of mammals (Reeves et al., 1982; Smale and Butler, 1994; Willford et al., 1990), and (3) not dependent on pH (%CO_2) (Fig. 1). The Bohr effect was slightly lower than previously reported for the bar-headed goose (Black and Tenney, 1980), but similar to that of other birds and mammals (Baumann and Baumann, 1977; Danzer and Cohn, 1967; Grispo et al., 2012; Lutz et al., 1973; Weber and Campbell, 2011), and was not temperature dependent. Hemoglobin concentration in this study was slightly higher and hematocrit slightly lower than values previously reported for bar-headed geese (Black and Tenney, 1980; Petschow et al., 1977; Scott and Milsom, 2007), and likely reflects hydration levels, etc. of the birds in different studies.

While the calculated temperature coefficient ($\Delta\log P_{50}/\Delta T$) of whole-blood of bar-headed geese in the present study was substantially greater than that of most other birds, including the

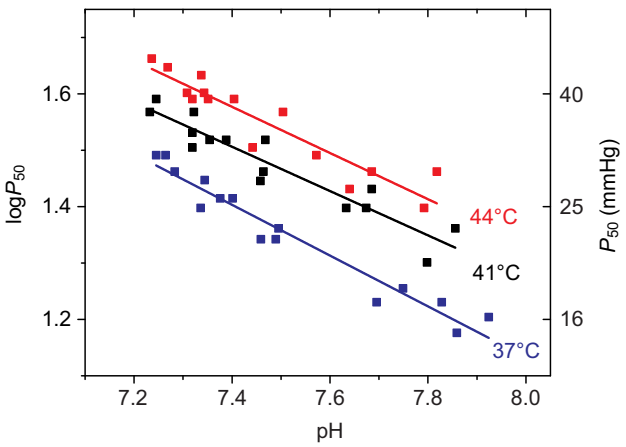

Fig. 1. The CO_2 Bohr effect of the whole blood of bar-headed geese at varying temperatures. The Bohr coefficient ($\Delta\log P_{50}/\Delta$pH) was −0.48±0.06 at 37°C, −0.42±0.02 at 41°C and −0.48±0.09 at 44°C over the full range studied (no significant differences, one-way ANOVA).

domestic goose [0.028 for the bar-headed *versus* 0.021 for the domestic goose, a difference of 25% (Danzer and Cohn, 1967)], when the actual shift in P_{50} per °C (1.84 mmHg °C^{-1}) for bar-headed goose blood is compared with that reported for the domestic goose, the result is slightly less than that in the Danzer and Cohn study (~2 mmHg °C^{-1}), but remains 25% higher than that reported in an earlier study by Wastl and Leiner (Wastl and Leiner, 1931). Although these discrepancies may be a result of the difference in the temperature ranges tested or techniques used, it remains unclear whether increased thermal sensitivity is unique to bar-headed goose Hb or is a common feature of geese Hb in general. Additional experiments on other goose species using the same methodology, as well as more detailed studies spanning the full range of the blood–O_2 equilibrium curve (and on isolated Hb) and taking into account additional allosteric modulators of Hb–O_2 affinity (fixed acids, organic phosphates, etc.) in bar-headed geese and other goose species, will be required to resolve these issues.

More relevant than the specific temperature coefficient, however, is the extent to which temperature and [CO_2] can affect O_2 delivery in the bar-headed goose during its high-altitude migration. Using our data in combination with previously published values of arterial and venous P_{O_2} from bar-headed geese at various levels of hypoxia (Black and Tenney, 1980; Scott and Milsom, 2007), we produced blood–O_2 equilibrium curves (expressed as O_2 content *versus* P_{O_2}) for bar-headed geese at altitudes of 6000 and 9000 m (Fig. 2). It has been demonstrated that bar-headed geese have an enhanced hypoxic ventilatory response to poikilocapnic hypoxia (due to a larger tidal volume, enhancing parabronchial ventilation) and that bar-headed geese experience a significant alkalosis in hypoxia (pH ~7.2) (Scott and Milsom, 2007). Given the documented decreases in body temperature induced by hypoxia in birds in hypobaric hypoxia (Maginniss et al., 1997) and bar-headed geese in normobaric hypoxia (Scott et al., 2008), and particularly the reduction in caudal air sac temperature measured after rapid ventilation of extremely cold air in pigeons (Maginniss et al., 1997), we believe a reduction in temperature at the blood–gas barrier is possible in a high-flying bird. For the sake of discussion, we consider 37°C, only 4°C lower than baseline body temperature in this species, a conservative estimate of parabronchial temperature at altitude given the enhanced ventilation of extremely cold ambient air (−20°C). If so, our P_{50} measurements at 37°C and 1% CO_2 probably yield a reasonable estimate of the blood–O_2 equilibrium curve of blood at the blood–gas barrier in the lung. Conversely, as flapping flight is sustained in migrating bar-headed geese (Hawkes et al., 2011), heat and CO_2 will be added to capillary blood in the tissues, making the blood–O_2 equilibrium curve measured at 44°C and 7% CO_2 a reasonable estimate for blood in vessels draining locomotory muscle. Such values are not exceptional for animals during exercise (Bayly et al., 1989; Fuller et al., 2000; Irving, 1959; Mitchell et al., 2006; Schmidt-Nielsen et al., 1957; Taylor et al., 1998). Using the blood–O_2 equilibrium curve associated with cold, hypocapnic blood (as might be found in the lung) and warm, hypercapnic blood (as might be found in exercising muscle) to calculate arterial and venous O_2 contents, respectively, potential temperature- and pH-induced increases in O_2 loading and unloading were estimated at each altitude. Based on this analysis, temperature and pH effects acting on blood–O_2 affinity could increase O_2 delivery approximately twofold at migratory altitudes (6000–9000 m), compared with what would be delivered by blood at constant temperature and pH (Fig. 2). This doubling of blood O_2 extraction represents a significant improvement that likely contributes to sustaining the metabolically costly activity of flapping flight during high altitude migration.

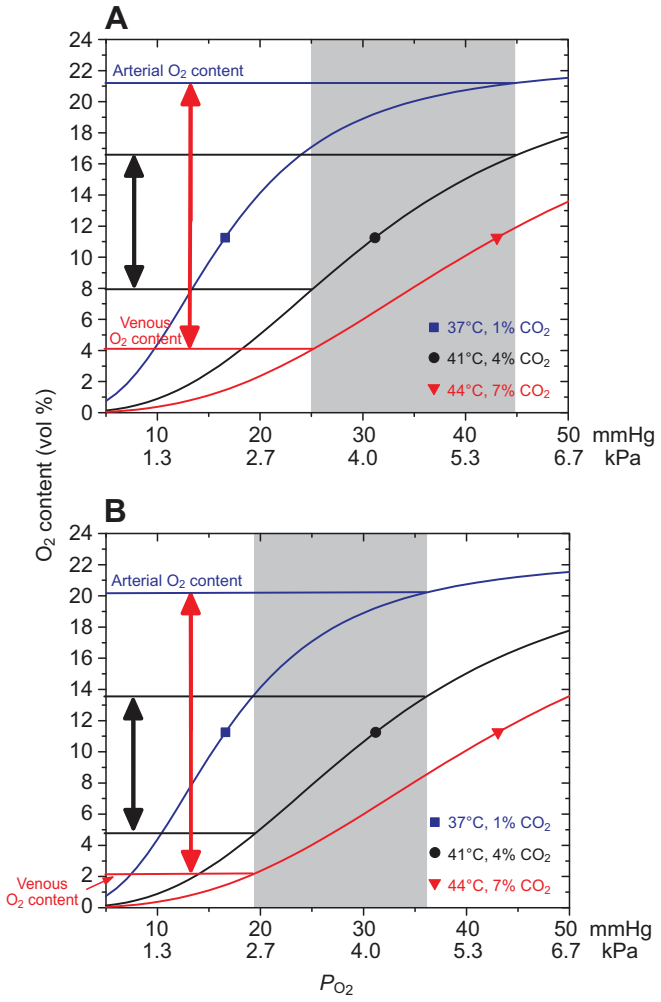

Fig. 2. Estimated blood–O_2 equilibrium curves (expressed as O_2 content *versus* P_{O_2}) at 6000 m (A) and 9000 m (B) assuming the measured P_{50} value from this study and a Hill coefficient of 2.8, as previously documented for the bar-headed goose (Black and Tenney, 1980; Petschow et al., 1977). The data point marked on each curve represents the mean P_{50} value measured in this study under the conditions indicated for each curve. The O_2 content was calculated from: [Hb]=17.1 g dl^{-1} (2.59 mmol l^{-1}) (this study) and arterial/venous P_{O_2} data (indicated by the right and left edge of the grey box, respectively) from bar-headed geese in hypoxia from previous studies (Black and Tenney, 1980; Scott and Milsom, 2007), where O_2 content (vol%)=(1.34 ml O_2 g^{-1} Hb) × [Hb] × S_{O2} + (0.003 × P_{O_2}). The potential temperature- and pH-induced increase in O_2 delivery [shift in arterial and venous O_2 content when using the left and right shifted curves, respectively (red arrow), compared with constant temperature (41°C) and pH (4% CO_2) (black arrow)] is marked for each altitude [6000 m (A) and 9000 m (B)]. Note that although the Hill coefficient may vary with saturation (Lutz, 1980), this difference in O_2 delivery (approximately twofold) is maintained when a higher Hill coefficient (~4) at high saturations and a low value (~1.5) at low saturations [reasonable values for those obtained for avian species (Lutz, 1980)] are assumed. Thus, we present our curves assuming a constant Hill coefficient of 2.8.

In addition to their role in oxygen delivery, temperature and pH effects on Hb may also play a role in heat dissipation in migrating birds (Giardina et al., 1990). Increased thermal sensitivity of Hb will also improve O_2 loading at nasal and buccal mucosa and ocular surfaces, believed to aid in supplying O_2 to the brain in pigeons (Bernstein et al., 1984; Pinshow et al., 1985). Interestingly, calculations of the magnitude of the enhanced brain P_{O_2} and O_2

saturation achieved *via* this mechanism in birds at an altitude of 7000 m (Pinshow et al., 1985) are similar to those determined for increased O_2 delivery to exercising muscle in the present study. Increased temperature dependence should also preserve O_2 unloading in a species with an intrinsically high affinity Hb and unremarkable Bohr effect, as is the case in the bar-headed goose.

In summary, these data indicate that hypocapnic, cold blood at the blood–gas barrier in the lung would significantly enhance O_2 loading and that hypercapnic, warm blood in exercising muscle would significantly augment O_2 unloading in the bar-headed goose during flight at high altitude. These results add to a recently expanding body of literature on the thermal sensitivity of Hbs. While reductions in temperature dependence may be adaptive to the Hb of several heterothermic vertebrates (Weber and Campbell, 2011), increases in thermal sensitivity may be adaptive in aquatic environments with large daily temperature fluctuations (Wilhelm and Weber, 1983) or in high-altitude flight. There are numerous mechanisms by which the high thermal sensitivity of bar-headed goose Hb may be produced (Weber and Campbell, 2011). Further studies will be required to determine *in vivo* O_2, CO_2, lactate and pH levels and body temperatures in various anatomical compartments during high-altitude migration (or under simultaneous conditions of hypobaric hypoxia and reduced temperatures) in this species. Such knowledge will facilitate a more complete analysis of the contribution of O_2–Hb equilibrium curve properties to the remarkable physiology of the high-flying bar-headed goose.

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AUTHOR CONTRIBUTIONS

J.U.M. and W.K.M. conceived and designed the experiments and prepared the manuscript. J.U.M. performed the experiments and data analysis.

COMPETING INTERESTS

No competing interests declared.

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