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Flying high: A theoretical analysis of the factors limiting exercise performance in birds at altitude☆

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Abstract

The ability of some bird species to fly at extreme altitude has fascinated comparative respiratory physiologists for decades, yet there is still no consensus about what adaptations enable high altitude flight. Using a theoretical model of O_2 transport, we performed a sensitivity analysis of the factors that might limit exercise performance in birds. We found that the influence of individual physiological traits on oxygen consumption ($\dot{V}_{O_2}$) during exercise differed between sea level, moderate altitude, and extreme altitude. At extreme altitude, haemoglobin (Hb) O_2 affinity, total ventilation, and tissue diffusion capacity for O_2 ($D_{T_{O_2}}$) had the greatest influences on $\dot{V}_{O_2}$; increasing these variables should therefore have the greatest adaptive benefit for high altitude flight. There was a beneficial interaction between $D_{T_{O_2}}$ and the P_{50} of Hb, such that increasing $D_{T_{O_2}}$ had a greater influence on $\dot{V}_{O_2}$ when P_{50} was low. Increases in the temperature effect on P_{50} could also be beneficial for high flying birds, provided that cold inspired air at extreme altitude causes a substantial difference in temperature between blood in the lungs and in the tissues. Changes in lung diffusion capacity for O_2, cardiac output, blood Hb concentration, the Bohr coefficient, or the Hill coefficient likely have less adaptive significance at high altitude. Our sensitivity analysis provides theoretical suggestions of the adaptations most likely to promote high altitude flight in birds and provides direction for future in vivo studies.

Keywords: Birds; Blood; Haemoglobin; Comparative physiology; Control of breathing; Hypoxic responses; Exercise; Gas exchange; Diffusing capacity; Models; Mathematical

1. Introduction

Oxidative metabolism provides the energy for cellular processes in the majority of living organisms, yet many animals can survive and reproduce in environments with very little oxygen. Living in these environments generally requires many physiological, cellular, and molecular adaptations that match oxygen supply and oxygen demand. On one hand, there are examples

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in all vertebrate classes of species that reduce oxygen demand in hypoxia by suppressing total metabolism (Boutilier, 2001). On the other hand, some animals have a remarkable capacity to increase oxygen supply in hypoxia, and generally do so by increasing the flux capacity of oxygen transport pathways (Hochachka, 1985). This strategy is especially important in species that exercise in hypoxic environments, and there is perhaps no better example of animals that perform high intensity exercise during hypoxia than birds flying at altitude. Indeed, oxygen consumption may increase 10–20-fold during flight (Butler et al., 1977; Ward et al., 2002; Peters et al., 2005), and some birds are known to fly at exceptionally high altitudes. Bar-headed geese (*Anser indicus*) are perhaps the best known in this regard: this species flies over the Himalayan mountains twice a year on its migratory route between Tibet and India and has been sighted flying above the summit of Mount Everest (8848 m above sea level) (Swan, 1970).

How species such as the bar-headed goose fly at altitudes that can render many mammals comatose (Tucker, 1968) remains a mystery. It seems reasonable, however, that an enhanced oxygen flux capacity along the path from environment to mitochondria plays a substantial role. This oxygen cascade consists of many steps, including ventilation, diffusion of O_2 into the blood, circulation of O_2 throughout the body, and diffusion from the blood to the tissue mitochondria. Important adaptations in high altitude fliers could exist at multiple steps in this cascade. In fact, if we consider symmorphosis as a null hypothesis, adaptations to enhance O_2 flux should exist at all steps in the cascade (Weibel et al., 1991). Substantial research has explored some of these steps in high altitude birds, as will be discussed in the following sections, but others remain largely unexplored.

The present study was undertaken to re-address the question of how some birds are able to fly at extreme altitude. The potential physiological adaptations supporting high altitude flight have attracted a great deal of attention in the past (Faraci, 1991) and this research will first be reviewed briefly (highlighted in Table 1). A theoretical sensitivity analysis will then be presented that explores the possible factors that could limit exercise performance in birds at high altitude. The primary objective of this analysis is to quantitatively assess potential adaptations to high altitude hypoxia in birds, and to theoretically assess the possible influence of changes in different physiological variables on exercise performance.

Table 1
Potential physiological adaptations for high altitude bird flight

Physiological trait	Reference
Respiratory system	
High maximum ventilation rates	Tucker (1968)
Reduced sensitivity of ventilation to hypocapnia/alkalosis	Scheid (1990)
Small diffusion barrier	Maina and King (1982)
Reduced pulmonary vasoconstriction	Faraci et al. (1984a)
Circulatory system	
High cardiac output	Black and Tenney (1980)
High Hb-O_2 affinity	Petschow et al. (1977)
Enhanced blood flow to brain and heart	Faraci et al. (1984b)
Effective extracellular pH regulation	Dodd and Milsom (1987)
Tissues	
Greater capillary density	Fedde et al. (1985)
Greater abundance of mitochondria	Fedde et al. (1985)
Effective intracellular pH regulation	Weinstein et al. (1985)

The above list represents previous suggestions of high altitude adaptations in birds, and some of these traits remain to be thoroughly explored in vivo. See text for details.

1.1. The respiratory system and oxygen transport

The bird lung is unique amongst the lungs of air-breathing vertebrates, with a blood flow that is crosscurrent to gas flow, and a gas flow that occurs unidirectionally through rigid parabronchioles. As such, bird lungs are inherently more efficient than the lungs of other air-breathing vertebrates (Piiper and Scheid, 1972, 1975). While this may partially account for the greater hypoxia tolerance of birds in general when compared to mammals (cf. Scheid, 1990), its presence in all birds excludes the crosscurrent lung as a possible adaptation specific to high altitude fliers. Similarly, an extremely small diffusion distance across the blood–gas interface compared to other air breathers seems to be a characteristic of all bird lungs, and not just those of high fliers (Maina and King, 1982; Powell and Mazzone, 1983; Shams and Scheid, 1989). Partly because of this small diffusion distance, the inherent O_2 diffusion capacity across the gas–blood interface ($D_{L_{O_2}}$) is generally high in birds (Shams and

Scheid, 1989), although other factors can conspire to decrease the efficiency of lung to blood oxygen transfer. For example, pulmonary vasoconstriction and/or ventilation–perfusion heterogeneity may occur in some birds during hypoxia (Faraci et al., 1984a; Schmitt et al., 2002) and could limit O_2 exchange. Interestingly, pulmonary vasoconstriction does not appear to increase during hypoxia in bar-headed geese (Faraci et al., 1984a). This may be a significant advantage during combined exercise and severe hypoxia, and suggests that regulation of lung blood flow could be important in high altitude birds.

Birds generally increase ventilation during hypoxia, primarily (but not exclusively) due to increases in breathing frequency (Bouverot et al., 1976; Colacino et al., 1977; Brackenbury et al., 1982; Powell et al., 2000). A heightened capacity to increase ventilation during severe hypoxia might benefit high altitude birds, because it could reduce the difference between inspired P_{O_2} and arterial P_{O_2} (Tucker, 1968; Shams and Scheid, 1989). Data in support of this suggestion have unfortunately been inconclusive. For example, whereas bar-headed geese can increase ventilation by nearly 10-fold during severe poikilocapnic hypoxia (3% O_2, equivalent to 11,580 m elevation) (Black and Tenney, 1980) and during treadmill exercise in moderately severe poikilocapnic hypoxia (7% O_2, 7620 m) (Fedde et al., 1989), pekin ducks (*Anas platyrhynchos*) can make similar ventilatory adjustments (Black and Tenney, 1980; Kiley et al., 1985) even though they are not known to fly at extreme altitudes.

While these data suggest that ventilation might not limit oxygen consumption during severe hypoxia, and that changes in ventilatory control may not contribute significantly to high altitude performance in birds, several important issues are still unresolved. Running and swimming in ducks and geese result in only 2–3-fold increases in oxygen consumption (Kiley et al., 1985; Butler and Turner, 1988; Fedde et al., 1989), compared to the 15–20-fold increases seen during flight (Ward et al., 2002), and differences in the capacities of the respiratory systems in low and high altitude birds may only become apparent when ventilatory demands increase to those seen during flight. Furthermore, hyperventilation at altitude is associated with a respiratory hypocapnia/alkalosis, which tends to inhibit breathing. If high altitude birds are less sensitive to changes in CO_2/pH they might be better able to sustain increases in ventilation during severe hypoxia coupled with exercise. The CO_2/pH sensitivity of ventilation is commonly assessed by comparing the isocapnic and poikilocapnic hypoxic ventilatory responses; however, the isocapnic ventilatory responses to hypoxia of both low and high altitude birds have not been compared (but see Powell et al., 2000). In this regard, the ventilatory response in high altitude birds may also depend on their capacity to maintain intracellular pH during alkalosis (Weinstein et al., 1985), or to buffer changes in extracellular pH due to hyperventilation (Dodd and Milsom, 1987). It therefore remains to be conclusively determined whether high altitude fliers have a greater capacity to increase ventilation during severe hypoxia.

1.2. The circulatory system and oxygen transport

After diffusing into the blood in the lungs, oxygen is primarily circulated throughout the body bound to haemoglobin. A high cardiac output is therefore important for exercise at high altitude to supply the working muscle with adequate amounts of O_2. Indeed, animals selectively bred for exercise performance have higher maximum cardiac outputs (Hussain et al., 2001), as do species that have evolved for exercise performance (Hoppeler and Weibel, 1998). Whether cardiac output limits exercise performance per se, however, is less clear; other factors may limit intense exercise, and in more athletic species (or individuals) cardiac output may be higher simply out of necessity (Wagner, 1996a). Excessive cardiac output may even be detrimental if blood transit times in the lungs or tissues are substantially reduced. Unfortunately, very little is known about cardiac performance in high flying birds. Both the high altitude bar-headed goose and the low altitude pekin duck can increase cardiac output at least five-fold during hypoxia at rest (Black and Tenney, 1980), but no comparison of maximum cardiac performance has been made between high and low altitude birds.

Changes in blood flow distribution during hypoxia have received a great deal of attention in birds. In general, hypoxia-induced hyperventilation causes hypocapnia and respiratory alkalosis. Because cerebral blood flow (CBF) is primarily regulated by the P_{CO_2}/pH of the cerebrospinal fluid, high altitude results in cerebral vasoconstriction and a severe reduction in brain oxygen delivery in most mammals (Faraci

and Fedde, 1986). Interestingly, whereas the effects of hypercapnia/acidosis on CBF appears to be similar in birds and mammals, CBF in birds appears to be insensitive to the effects of hypocapnia/alkalosis, unlike in mammals (Grubb et al., 1978; Faraci and Fedde, 1986). However, the ability to maintain CBF during hypocapnia/alkalosis is thought to be present in all birds, so differences in CBF regulation are unlikely to be an adaptation specific to flight at extreme altitudes; indeed, cerebral oxygen delivery is maintained and is equivalent in both bar-headed geese and pekin ducks during severe hypoxia (Faraci et al., 1984b).

Not only must oxygen delivery be maintained to the brain, it must also increase substantially to the heart and pectoral muscle during flight at extreme altitudes. A portion of this increase in O_2 delivery arises from redistributing blood flow away from the splanchnic region, the kidneys, and the skin, towards the exercising muscle; however, the contribution of flow redistribution to the large increase in $\dot{V}_{O_2}$ during flight is probably small. This suggestion comes from research comparing the impact of redistributing blood flow to working muscle between sedentary and trained individuals or species: for individuals/species with a high $\dot{V}_{O_2MAX}$, the impact of supplying more blood to the muscles is diminished (Hochachka, 1985). Instead, high altitude species have frequently adapted to hypoxia by increasing the affinity of their haemoglobin (Hb) for O_2 (Snyder, 1985; Samaja et al., 2003). This is the case for many high flying birds (Petschow et al., 1977) whose increased Hb-O_2 affinity is frequently based on only one or a few amino acid point mutations (Braunitzer and Hiebl, 1988; Weber et al., 1988, 1993; Zhang et al., 1996; Weber and Fago, 2004). As a result, species such as the bar-headed goose can sustain greater oxygen delivery to muscles than low altitude birds during hypoxia, because at any given blood P_{O_2} their blood oxygen concentration will be higher (Faraci et al., 1984b).

Even though haemoglobin with a lower P_{50} increases oxygen loading across the lungs, and can result in higher tissue oxygen delivery, the absolute benefit of a low P_{50} on oxygen consumption ($\dot{V}_{O_2}$) is unclear. This is primarily because a low P_{50} inhibits oxygen unloading at the tissues, so it has been suggested that other characteristics of Hb that facilitate oxygen unloading may be important in altitude-adapted animals (Samaja et al., 2003). Previous suggestions include variations in the Bohr (CO_2/pH) effect on Hb-O_2 affinity and the nature of Hb-O_2 cooperativity. It is notable in this regard that the Bohr effect in bar-headed geese is less than in other waterfowl (Liang et al., 2001), suggesting that traits other than Hb biochemistry may increase O_2 unloading in high flying birds.

The concentration of Hb in the blood can also influence oxygen transport at altitude. Increased Hb content often accompanies altitude acclimatization in some mammals. Blood Hb concentration is generally the same in low and high altitude birds (Black and Tenney, 1980), however, and acclimation to hypoxia increases blood Hb in low altitude birds but not in high altitude birds. These results suggest that blood Hb concentration is not adaptive for high altitude flight.

1.3. Flight muscle and oxygen transport

Once oxygenated blood is circulated to the tissues, O_2 moves to the tissue mitochondria, the site of oxidative phosphorylation and oxygen consumption. Transport of oxygen from the blood to the mitochondria involves several steps. Oxygen must first dissociate from Hb and diffuse through the various compartments of the blood, but in both birds and mammals the conductances of these steps are high, and are unlikely to impose much of a limitation to O_2 transport (Phu et al., 1986). In contrast, diffusion across the vascular wall and through the extracellular spaces is thought to provide the most sizeable limitation to O_2 transport (Wittenberg and Wittenberg, 2003). Consequently, the size of the capillary–muscle fibre interface is an extremely important determinant of a muscle's aerobic capacity (Hepple, 2000; Mathieu-Costello, 2001). Finally, oxygen diffuses across the muscle fibre membrane and moves through the cytoplasm until it associates with cytochrome *c* oxidase, the O_2 acceptor in the mitochondrial electron transport chain. Myoglobin probably assists intracellular O_2 transport, so diffusion through the muscle likely provides very little resistance to O_2 flux (Gayeski and Honig, 1988; Wittenberg and Wittenberg, 2003).

Physiological traits that increase the oxygen diffusion capacity of muscle could be important for flight at extreme altitude in birds. Because the capillary–muscle fibre interface appears to provide the greatest resistance to oxygen flux, increases in flight muscle and

heart muscle capillarization could increase maximum $\dot{V}_{O_2}$ in high altitude birds. This is indeed the case in the flight muscle of finches, as those living at moderate altitude have higher capillarization than those living at sea level (Mathieu-Costello et al., 1998). Similar differences appear to exist between extreme altitude and sea level species. In a preliminary qualitative study, bar-headed geese had greater capillary density in their pectoral muscle than pekin ducks (Fedde et al., 1985). Furthermore, bar-headed geese reared in normoxia had greater gastrocnemius (leg) muscle capillarity than Canada geese (*Branta canadensis*), and this difference persisted even if Canada geese were reared in moderate hypoxia (Snyder et al., 1984). These results cannot necessarily be generalized to the flight muscle, however, due to the known differences in fibre-type composition. Myoglobin contents of the pectoral, cardiac, and gastrocnemius muscles are equivalent between bar-headed geese and other low-flying waterfowl (Snyder et al., 1984; Saunders and Fedde, 1991), supporting the idea that the sarcoplasm does not limit oxygen flux during exercise in hypoxia. Regardless, there appears to be at least some evidence suggesting that the oxygen diffusion capacity of the muscle (specifically the area of the capillary–muscle fibre interface) may be enhanced in high altitude fliers.

In addition to the diffusive pathways for oxygen flux in the muscle, oxygen consumption is influenced by certain characteristics of mitochondrial metabolism in the muscle. Both the maximum aerobic capacity and the affinity of mitochondria for oxygen can influence the O_2 consumption rate, so adaptation to high altitude could increase tissue mitochondria content or mitochondrial O_2 affinity (Hochachka, 1985; St-Pierre et al., 2000; Mathieu-Costello, 2001). Additionally, altitude adaptation could increase the activity of enzymes involved in aerobic metabolism (Hochachka, 1985; Sheafor, 2003). Unfortunately, very little data exist examining biochemical aspects of metabolism in high flying birds. One exception to this is the qualitative study by Fedde et al. (1985), where bar-headed geese were found to have larger more abundant mitochondria than pekin ducks in the pectoral muscle. This suggests that the high flying bar-headed geese have a greater aerobic capacity than other waterfowl, which may significantly increase maximum $\dot{V}_{O_2}$ during severe hypoxia. This difference is substantial and is superimposed on the already high aerobic capacity of bird flight muscle (Turner and Butler, 1988; Mathieu-Costello et al., 1998).

1.4. How do birds fly high?

From the above discussion it is obvious that the mechanistic bases of the ability of some bird species to fly at extreme altitude is poorly understood. The adaptive benefit of high haemoglobin oxygen affinity is well established, but its relative importance is unknown. Some evidence suggests that traits increasing oxygen diffusion capacity in flight muscle are adaptive in high fliers as well, but the adaptive significance of differences in the respiratory and cardiovascular systems of high altitude fliers is not clear. The remainder of this study will assess these possibilities using theoretical sensitivity analysis, and will explore potential adaptations for high altitude flight in birds.

2. Methods

2.1. Theoretical model of O_2 transport

This model has a similar structure to previous theoretical analyses of oxygen transport in mammals and reptiles (Wagner, 1996b; Wang and Hicks, 2002, 2004), but is altered to accommodate the crosscurrent arrangement of the avian lung (Fig. 1). Oxygen transport at the lung is modelled using parabronchial 'tubes' containing perfectly mixed gas, which contact a finite number of blood capillaries along their length at right angles. When gas enters the parabronchi and encounters the first lung capillary, oxygen diffuses from the parabronchi into the blood, as dictated by the following equation (analogous to Fick's diffusion equation):

$$\frac{dC_{cap_{O_2}}}{dt} = \frac{D_{L_{O_2}}}{t_{cap}\dot{Q}_{cap}}(P_{para_{O_2}} - P_{cap_{O_2}}) \quad (1)$$

where $dC_{cap_{O_2}}/dt$ is the rate of change of the instantaneous O_2 concentration in the capillary ($C_{cap_{O_2}}$), $D_{L_{O_2}}$ the diffusion conductance of the lung for O_2, t_{cap} the total capillary transit time, $\dot{Q}_{cap}$ the blood flow rate through the capillary, and $P_{para_{O_2}}$ and $P_{cap_{O_2}}$ are the instantaneous P_{O_2} in the parabronchi and the capillary, respectively. The O_2 tension of the gas encountering the second capillary is reduced, and is calculated by

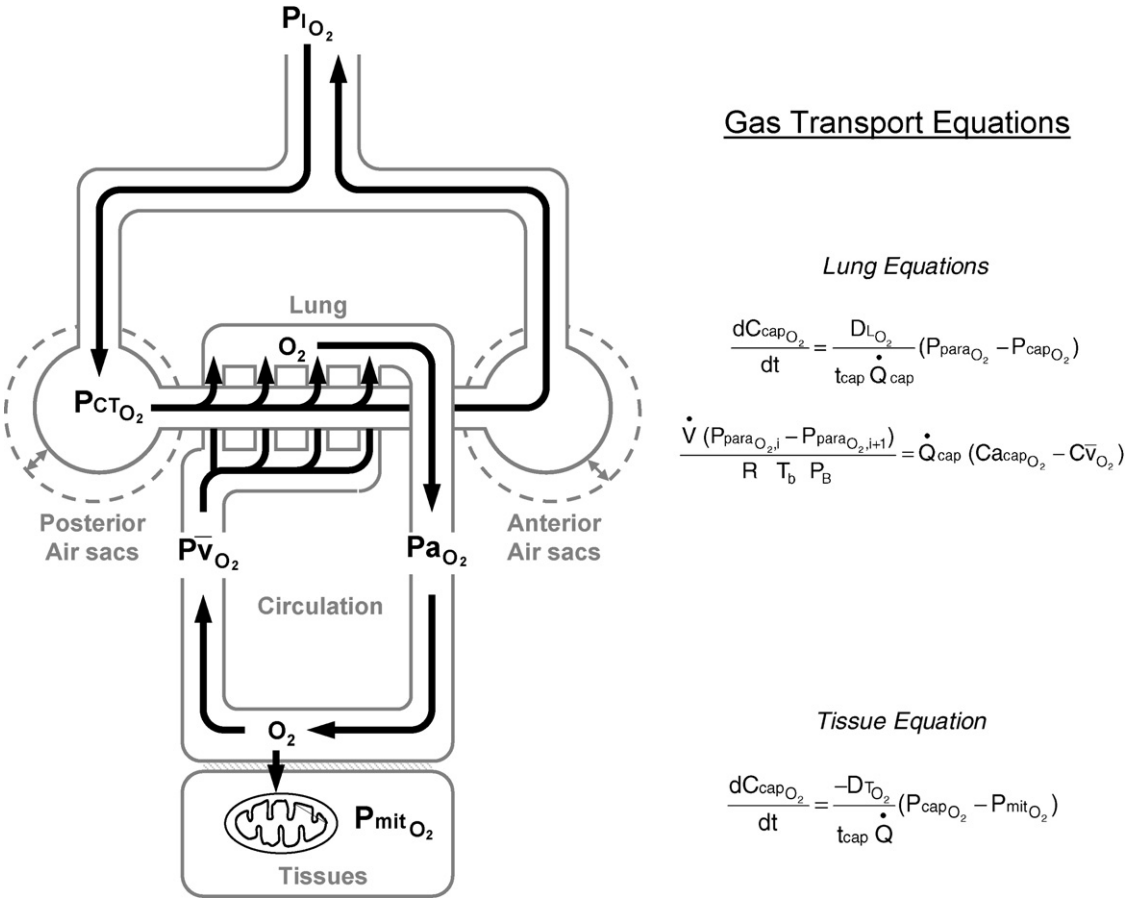

Fig. 1. Schematic of the oxygen transport pathway in birds. The crosscurrent parabronchial lung is unidirectionally ventilated by air sacs, and oxygen diffuses into blood capillaries from air capillaries (not shown) all along the length of the parabronchi. Oxygen is then circulated in the blood, and diffuses to mitochondria in the tissues. The rate of oxygen transport at both the lungs and tissues can be calculated using the Fick equation, and the amount of O_2 transferred from the lungs into the blood can be calculated using an oxygen conservation equation. See Section 2 for definitions of all variables, as well as other equations used in the model and additional details.

mass conservation.

$$\frac{\dot{V}(P_{\mathrm{para}_{O_2,1}} - P_{\mathrm{para}_{O_2,2}})}{RT_b P_B} = \dot{Q}_{cap}(\mathrm{Ca}_{\mathrm{cap}_{O_2}} - \mathrm{C\bar{v}}_{O_2}) \quad (2)$$

where ventilation ($\dot{V}$) is assumed constant along the parabronchiole (i.e., a respiratory quotient of 1), $P_{\mathrm{para}_{O_2,1}}$ and $P_{\mathrm{para}_{O_2,2}}$ the $P_{\mathrm{para}_{O_2}}$ at the first and second capillary, R the gas constant, T_b the avian body temperature in Kelvin (314 K), P_B the barometric pressure, and $\mathrm{Ca}_{\mathrm{cap}_{O_2}}$ and $\mathrm{C\bar{v}}_{O_2}$ (mixed venous C_{O_2}) are the oxygen concentrations leaving and entering the first capillary, respectively. Eqs. (1) and (2) are then repeated for the second capillary, and all subsequent capillaries, and the resulting arterial P_{O_2} (Pa_{O_2}) is calculated from arterial O_2 content (Ca_{O_2}, the average value from all the $\mathrm{Ca}_{\mathrm{cap}_{O_2}}$ leaving the capillaries) using Eq. (4). The arterial P_{O_2} is independent of the arbitrary number of capillaries used in the model (above approximately 20 capillaries), so the number of capillaries along the length of the parabronchi was set at 50. $\dot{Q}_{cap}$ was therefore set as the total cardiac output ($\dot{Q}$) divided by 50.

Oxygen transport at the tissues is modelled using capillaries containing perfectly mixed blood. Blood

entering the capillary network has the Pa_{O_2} determined from the lung calculations above, and loses oxygen to the surrounding cells as determined by an equation similar to Eq. (1) above.

$$\frac{dC_{cap_{O_2}}}{dt} = \frac{-D_{T_{O_2}}}{t_{cap}\dot{Q}}(P_{cap_{O_2}} - P_{mit_{O_2}}) \quad (3)$$

where $D_{T_{O_2}}$ is the diffusion conductance of the tissues for O_2, and $P_{mit_{O_2}}$ is the instantaneous P_{O_2} at the mitochondria (set at zero, see below). The mixed venous P_{O_2} ($P\bar{v}_{O_2}$) is simply the P_{O_2} of blood leaving the tissue capillaries. When integrated over the length of the capillaries, t_{cap} cancels out of Eqs. (1) and (3), so need not be known.

Three additional equations, in addition to the above three primary equations describing oxygen transport, are important for the model. The first is the Hill equation, which describes the relationship between O_2 concentration and O_2 tension in the blood.

$$C_{O_2} = 4C_{Hb}\frac{P_{O_2}^n}{P_{O_2}^n + P_{50}^n} \quad (4)$$

where C_{Hb} is the concentration of haemoglobin (Hb) in the blood, n the Hill coefficient (describes the cooperativity of Hb-O_2 binding), and P_{50} is the O_2 tension at which Hb is 50% saturated with oxygen.

The second additional equation describes the Bohr effect on P_{50}.

$$P_{50} = P_{50i} \times 10^{\phi\,\Delta pH(a-v)} \quad (5)$$

where P_{50i} is the P_{50} before the Bohr effect, ϕ the Bohr coefficient, and $\Delta pH(a-v)$ is the arterio-venous pH difference. Using this equation, P_{50} increases at the tissues so that the effects of CO_2/pH could be accounted for in the model.

The third additional equation is an empirical relationship that accounts for the effect of ventilation on the P_{O_2} in the caudal thoracic air sac ($P_{CT_{O_2}}$; approximates the P_{O_2} entering the parabronchi): as $\dot{V}$ increases, dead space gas contributes less to the $P_{CT_{O_2}}$, and so the difference between $P_{I_{O_2}}$ and $P_{CT_{O_2}}$ decreases.

$$P_{CT_{O_2}} = P_{I_{O_2}} - 33.2e^{-0.43\,\dot{V}} \quad (6)$$

This relationship was derived using data from the available studies on pekin ducks that included normoxia and various degrees of hypoxia (Colacino et al., 1977; Shams and Scheid, 1989, 1993), and described most of the variation in their data ($R^2 = 0.95$). $P_{CT_{O_2}}$ is used in a somewhat similar way to alveolar P_{O_2} in previous theoretical models (Wagner, 1996b; Wang and Hicks, 2004), and allows the effect of $\dot{V}$ on the difference between $P_{I_{O_2}}$ and $P_{CT_{O_2}}$ to be accounted for in the sensitivity analysis (described below).

2.2. Solution of the model

The model used data from the literature on pekin ducks when available to reproduce in vivo conditions near $\dot{V}_{O_2MAX}$ during normoxia ($P_{I_{O_2}}$ of 150 Torr), moderate hypoxia (84 Torr), and severe hypoxia (30 Torr) (Table 2). Several measured parameters, namely $P_{I_{O_2}}$, $\dot{V}$, $\dot{Q}$, C_{Hb}, P_{50}, ϕ, $\Delta pH(a-v)$, and n, were put directly into the model, after which $D_{L_{O_2}}$ and $D_{T_{O_2}}$ were calculated to reproduce the measured in vivo Pa_{O_2}, $P\bar{v}_{O_2}$, and $\dot{V}_{O_2}$. Given the assumptions below, the model solves

Table 2
Literature values used in model calculations

Variable	Normoxia (sea level)	Moderate Hypoxia (3500 m)	Severe Hypoxia (10,000 m)
$P_{I_{O_2}}$ (Torr)	150	84	30
$\dot{V}$ (BTPS, L/min)	3.0	5.0	4.5
$\dot{Q}$ (L/min)	1.2	1.4	1.8
Pa_{O_2} (Torr)	98	60	26
$P\bar{v}_{O_2}$ (Torr)	43	33	5
C_{Hb} (mM)	2.3	2.3	2.3
P_{50} (Torr)	40	40	40
ϕ	0.4	0.4	0.4
$\Delta pH(a-v)$	0.1	0.1	0.1
n	2.8	2.8	2.8
T_B (°C)	41	41	41
$\dot{V}_{O_2}$ (mmol/min)	4.9	5.5	2.0
$D_{L_{O_2}}$ (mmol/Torr min)[a]	0.075	0.250	0.500
$D_{T_{O_2}}$ (mmol/Torr min)[a]	0.080	0.120	0.135

$P_{I_{O_2}}$, inspired O_2 tension; $\dot{V}$, total ventilation; $\dot{Q}$, cardiac output; Pa_{O_2}, arterial O_2 tension; $P\bar{v}_{O_2}$, mixed venous O_2 tension; C_{Hb}, haemoglobin (Hb) concentration; P_{50}, P_{O_2} at half-saturation of Hb with O_2; ϕ, Bohr coefficient; $\Delta pH(a-v)$, arterio-venous pH difference; n, Hill coefficient; T_B, body temperature; $\dot{V}_{O_2}$, oxygen consumption rate; $D_{L_{O_2}}$, O_2 diffusion capacity in the lung; $D_{T_{O_2}}$, O_2 diffusion capacity in the tissues. Literature sources for most starting data: normoxia and moderate hypoxia, Kiley et al. (1985); severe hypoxia, Black and Tenney (1980). Other starting data came from Black and Tenney (1980) (P_{50} and ϕ) and Weber and Fago (2004) (n), and T_B was set at the commonly accepted value for birds.

[a] $D_{L_{O_2}}$ and $D_{T_{O_2}}$ were calculated using all other literature values.

Eqs. (1)–(3) for the three unknown outcome variables Pa_{O_2}, $P\bar{v}_{O_2}$, and $P_{E_{O_2}}$ (P_{O_2} of expired gas), and then calculates $\dot{V}_{O_2}$ using two separate equations.

$$\dot{V}_{O_2} = \frac{\dot{V}(P_{I_{O_2}} - P_{E_{O_2}})}{RT_b P_B} \quad (7)$$

$$\dot{V}_{O_2} = \dot{Q}(Ca_{O_2} - C\bar{v}_{O_2}) \quad (8)$$

The model was solved iteratively using a program written in Matlab (Version 7) as follows. From a starting estimate of $P\bar{v}_{O_2}$, values for Pa_{O_2} and $P_{E_{O_2}}$ were calculated by integrating Eq. (1) then solving Eq. (2) ('lung equations') for each capillary in the lungs, as described above. From the determined value of Pa_{O_2}, Eq. (3) ('tissue equation') was integrated to calculate a new value for $P\bar{v}_{O_2}$. The lung and tissue equations were then repeated iteratively, using the outcome from each calculation as inputs in the next calculation, until a stable solution was reached (defined as the point when two successive calculations of Pa_{O_2} and $P\bar{v}_{O_2}$ were both within 0.1 Torr). Within the range of data reported in this study, $\dot{V}_{O_2}$ calculated by Eqs. (7) and (8) always agreed to within 0.1 mmol/min. Stable solution of the model generally took less than 10 iterations, and the final outcome was independent of the starting estimate of $P\bar{v}_{O_2}$ (it did, however, influence the number of required iterations).

2.3. Sensitivity analysis

From the starting point at each $P_{I_{O_2}}$, reproducing in vivo conditions near $\dot{V}_{O_2MAX}$ in the pekin duck, physiological parameters were then treated as independent variables in the model. Changing these independent variables over a wide range altered the values of the dependent outcome variables, Pa_{O_2}, $P\bar{v}_{O_2}$, and $P_{E_{O_2}}$, and therefore changed $\dot{V}_{O_2}$. As such, the effect on $\dot{V}_{O_2}$ of changing each physiological parameter individually was assessed. When some physiological parameters were changed drastically, stable solutions could not always be reached, so for these cases data are not reported.

Because high altitude is characterized by extremely low temperatures, the effect of temperature on P_{50} was also assessed in severe hypoxia. For this part of our analysis, cold inspired air was assumed to cool blood in the lungs by 10 °C, so haemoglobin in the blood was at 41 °C in the tissues and 31 °C in the lungs. Temperature was modelled to have a direct effect on P_{50} (in Torr/°C temperature change), such that the P_{50} decreased in the lungs and returned to normal at the tissues. The temperature effect was assessed over a range of temperature sensitivities from 0 to 2 Torr/°C, at starting P_{50} values of both 40 and 25 Torr.

2.4. Assumptions

The assumptions in our model are similar to those in other theoretical studies of oxygen transport (Wagner, 1996b; Wang and Hicks, 2002, 2004). Limitations on O_2 transport due to ventilation/perfusion heterogeneity in the lungs, metabolism/perfusion heterogeneity in the tissues, shunting of blood flow, and imperfect mixing of gas or blood are all treated as diffusion limitations to O_2 flux, and therefore contribute to the diffusion conductances in the lungs and tissues ($D_{L_{O_2}}$ and $D_{T_{O_2}}$). The 'tissues' are treated as one compartment, and it is assumed that total blood volume is constant in both the lung and tissue compartments. The respiratory quotient (RQ) is assumed to be 1.0, but the results of our analysis are unaltered if a different RQ is used (Wagner, 1996b). Whereas flight at altitude no doubt involves some non-steady-state changes in physiological variables, solution of our model only accounts for steady-state conditions. Finally, mitochondrial P_{O_2} was assumed to be zero, which is a reasonable assumption at $\dot{V}_{O_2MAX}$ (Gayeski and Honig, 1988; Wagner, 1996b). The data used in this study are from pekin ducks performing treadmill exercise near $\dot{V}_{O_2MAX}$ (Kiley et al., 1985; Butler and Turner, 1988) or for pekin ducks at their limit of hypoxia tolerance (Black and Tenney, 1980), so $P_{mit_{O_2}} \approx 0$ is likely a reasonable assumption. Although $\dot{V}_{O_2MAX}$ was not rigorously determined, these studies include all the required measurements and provided the best available data sets we could find.

3. Results

3.1. Oxygen transport in the lungs and tissues

In the crosscurrent arrangement of the avian lung, blood P_{O_2} varies in two dimensions (Fig. 2A). In our theoretical model, venous blood enters the lung capillaries and its P_{O_2} rises as it travels perpendicular to the

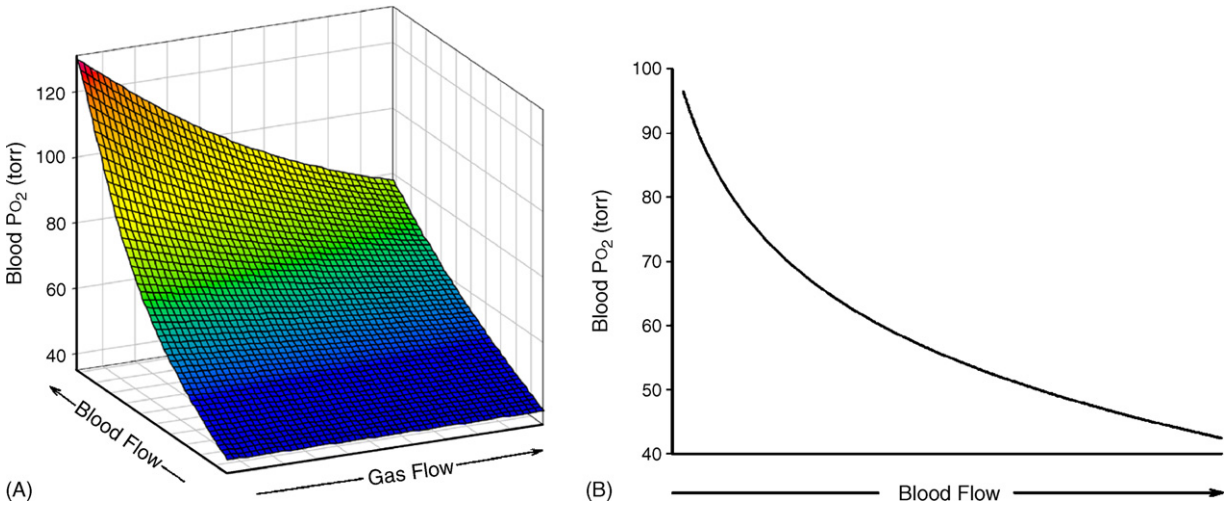

Fig. 2. Oxygen tensions in the lung (A) and tissue (B) capillaries during normoxia. In the crosscurrent avian lung, P_{O_2} varies in two dimensions: P_{O_2} increases along the path of blood flow through the lungs, but does not increase by as much at the end of the parabronchi as at the start (gas P_{O_2} decreases along the length of the parabronchi). In the tissues, blood P_{O_2} decreases continuously along the capillary length as O_2 diffuses to tissue mitochondria. Data were collected using the theoretical model of oxygen transport that is described in Section 2. To reach a solution, our model iterates between gas transport calculations in the lungs (A) and tissues (B) until a stable result is reached.

parabronchi, until it leaves the capillaries and becomes part of the arterial blood supply. Oxygen therefore diffuses into the blood and parabronchial O_2 levels fall as gas travels along the length of the parabronchi. As a result, blood P_{O_2} rises more if it travels through capillaries at the start of the parabronchi than at the end. Pa_{O_2} results from the mixing of blood leaving all the capillaries.

Oxygen transport in avian tissues is modelled in the same way as in other vertebrates (Fig. 2B). Blood enters the tissue capillaries with the same Pa_{O_2} that left the lungs. Oxygen then diffuses out of the blood continuously along the length of the capillaries. After leaving the tissues, blood has an oxygen tension of $P\bar{v}_{O_2}$ and is then circulated to the lungs. As described in Section 2, our model reaches a stable solution by iterating between gas transport calculations in the lungs (Fig. 2A) and tissues (Fig. 2B).

3.2. Effects of P_{50} on oxygen consumption

Altering the affinity of haemoglobin for oxygen (P_{50}) had different effects on oxygen consumption in normoxia, moderate hypoxia, and severe hypoxia (Fig. 3A). In normoxia, increasing P_{50} from the model's starting value (from literature values on pekin ducks near $\dot{V}_{O_2MAX}$) of 40 Torr, and thus decreasing Hb-O_2 affinity, increased $\dot{V}_{O_2}$ by up to 2% until approximately 60 Torr. Above 60 Torr, further increases in P_{50} decreased $\dot{V}_{O_2}$ slightly. Decreasing P_{50} from the model's starting value, however, decreased oxygen consumption sharply, such that at a P_{50} of 25 Torr $\dot{V}_{O_2}$ was reduced by 20%. A similar trend was observed in moderate hypoxia, except the highest $\dot{V}_{O_2}$ occurred at a P_{50} of 40 Torr, the starting point in the model. In contrast, $\dot{V}_{O_2}$ decreased sharply when P_{50} was increased in severe hypoxia, and $\dot{V}_{O_2}$ increased when P_{50} was decreased. The highest $\dot{V}_{O_2}$ in severe hypoxia occurred at a P_{50} of 10 Torr, at which point oxygen consumption was nearly 20% above the starting value of the model, and beyond this value $\dot{V}_{O_2}$ decreased sharply as P_{50} decreased further.

As discussed in Section 1, Hb-O_2 affinity is frequently higher in birds at high altitude. Bar-headed geese have a P_{50} of approximately 25 Torr, whereas many closely related waterfowl have a P_{50} of approximately 40 Torr (including pekin ducks, the species from which starting data for this study were derived). Many high altitude birds are already known to have a low P_{50}, so we performed the remaining sensitivity analyses at both 40 and 25 Torr. In doing so, we tested whether there is an interaction between P_{50} and other

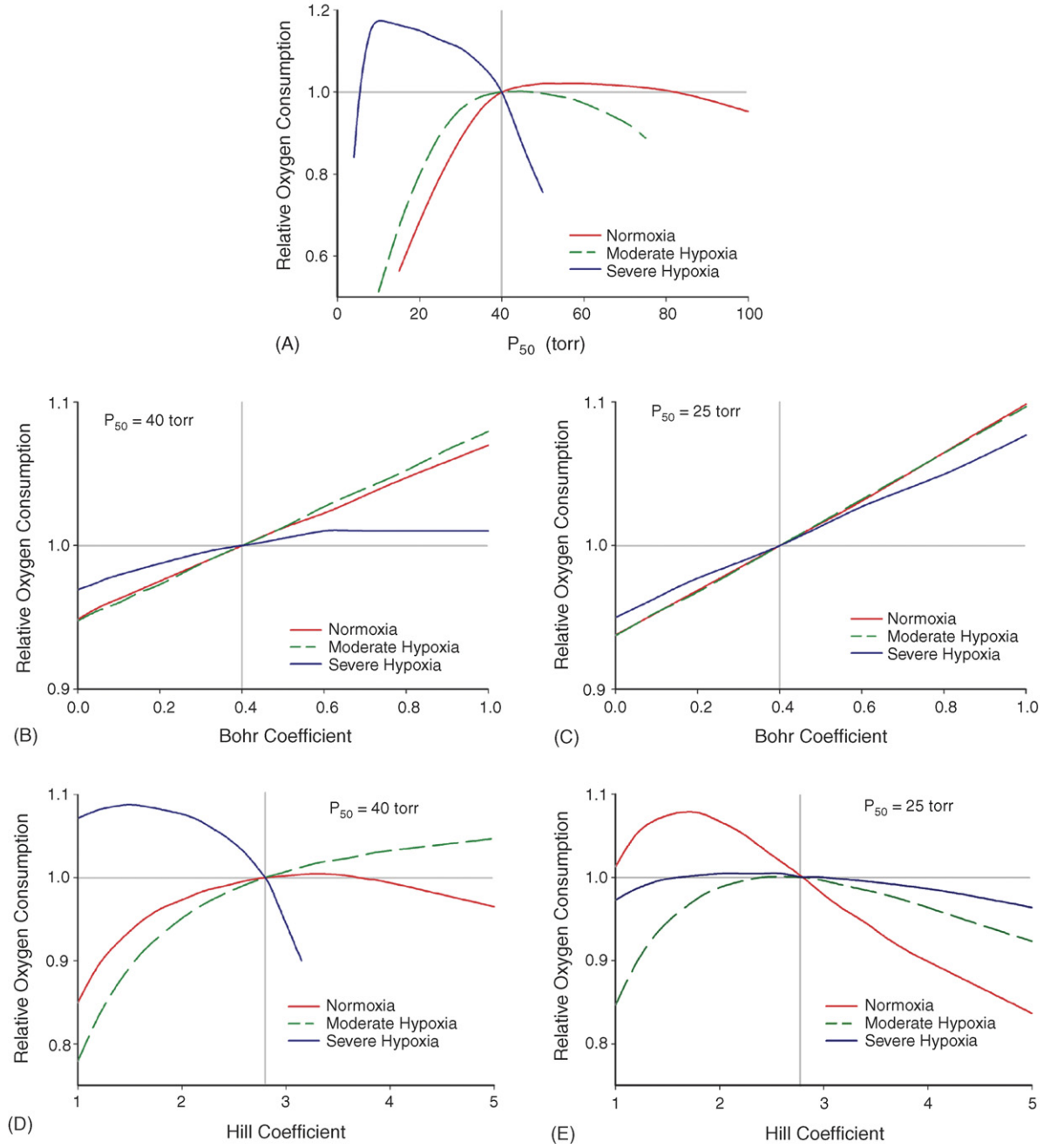

Fig. 3. The effects of varying different biochemical features of haemoglobin (Hb) on oxygen consumption during exercise in normoxia ($P_{I_{O_2}}$ of 150 Torr; red), moderate hypoxia (84 Torr; green dashed), and severe hypoxia (30 Torr; dark blue). (A) P_{50}, the P_{O_2} at 50% Hb saturation; (B and C) Bohr coefficient (ϕ); and (D and E) Hill coefficient (n) (see Section 2 for a mathematical description of each). In (B)–(E), the effects of each variable were assessed at the P_{50} of pekin ducks (40 Torr; B and D) as well as the P_{50} of bar-headed geese (25 Torr; C and E). Data were collected using the theoretical model of oxygen transport that is described in Section 2.

physiological variables, and consequently, whether the low P_{50} in high altitude species changes the adaptive benefit of different physiological traits.

3.3. Sensitivity analysis in normoxia

Altering biochemical properties of Hb other than P_{50} influenced oxygen consumption in normoxia. Altering the Bohr coefficient (ϕ) had a nearly linear effect for a P_{50} of both 40 and 25 Torr, such that increasing ϕ had a positive effect on $\dot{V}_{O_2}$ during exercise (Fig. 3B and C). The lower P_{50} enhanced the influence of ϕ, but in each case large alterations in ϕ resulted in no more than a 10% change in $\dot{V}_{O_2}$. Changing the Hill coefficient (n) had more variable effects on $\dot{V}_{O_2}$ (Fig. 3D and E). At a P_{50} of 40 Torr, increasing n from the starting value of 2.8 to about 3.7 enhanced $\dot{V}_{O_2}$ slightly (but <1%), after which $\dot{V}_{O_2}$ declined. Below 2.8, lowering n decreased $\dot{V}_{O_2}$ more sharply. The influence of the Hill coefficient was very different at a P_{50} of 25 Torr: the peak $\dot{V}_{O_2}$ shifted from an $n = 3.3$ at a P_{50} of 40 Torr, to $n = 1.7$ at a P_{50} of 25 Torr. The lower P_{50} therefore favoured less Hb-O_2 cooperativity in normoxia (i.e., a straighter Hb-O_2 saturation curve). On either side of 1.7, increasing or decreasing n reduced $\dot{V}_{O_2}$ sharply.

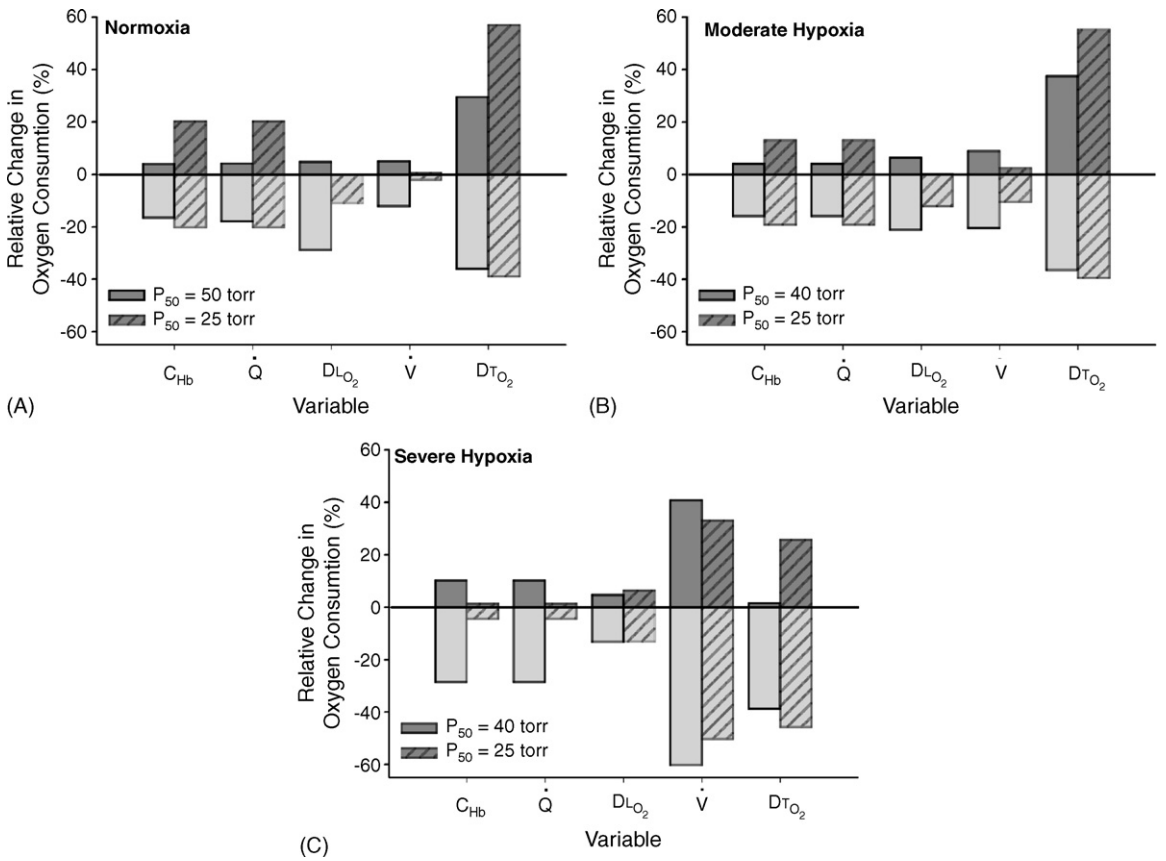

Fig. 4. The effects of a two-fold increase (dark bars) or decrease (light bars) in different physiological variables on oxygen consumption during exercise in (A) normoxia ($P_{I_{O_2}}$ of 150 Torr), (B) moderate hypoxia (84 Torr), and (C) severe hypoxia (30 Torr). The effects were assessed at the P_{50} of pekin ducks (40 Torr, solid bars) as well as the P_{50} of bar-headed geese (25 Torr, hatched bars). C_{Hb}, blood haemoglobin concentration; $\dot{Q}$, cardiac output; $D_{L_{O_2}}$, diffusion conductance of the lungs for O_2; $\dot{V}$, ventilation rate; $D_{T_{O_2}}$, diffusion conductance of the tissues for O_2. Data were collected using the theoretical model of oxygen transport that is described in Section 2. See Table 2 for starting values obtained from pekin ducks near $\dot{V}_{O_2MAX}$.

Data for other physiological variables are summarized for normoxia in Fig. 4A, which shows the effect of a two-fold increase or decrease of each variable on $\dot{V}_{O_2}$ during exercise. At a P_{50} of 40 Torr, increasing both cardiovascular variables (Hb concentration, C_{Hb}, and cardiac output, $\dot{Q}$) and respiratory variables (lung diffusion capacity, $D_{L_{O_2}}$, and ventilation rate, $\dot{V}$) had a small influence on $\dot{V}_{O_2}$ (<5%). Only increasing tissue diffusion capacity ($D_{T_{O_2}}$) had a substantial positive effect on $\dot{V}_{O_2}$ (30%). At a P_{50} of 25 Torr, the effect of increasing cardiovascular variables was enhanced (to 20%) and the influence of respiratory variables was reduced (<1%). Furthermore, the benefit of $D_{T_{O_2}}$ almost doubled (to 57%) at the lower P_{50}. In general, for normoxia as well as moderate hypoxia and severe hypoxia, decreasing physiological variables had a greater effect than increasing them, with the exception of C_{Hb}, $\dot{Q}$, and $D_{T_{O_2}}$ at the lower P_{50}. Furthermore, the effect of altering multiple physiological variables on $\dot{V}_{O_2}$ was additive rather than interactive (data not shown).

3.4. Sensitivity analysis in moderate hypoxia

The effects of the biochemical properties of Hb on $\dot{V}_{O_2}$ during exercise in moderate hypoxia were very similar to their effects in normoxia. The Bohr coefficient had a modest positive linear effect for both P_{50} values, and the lower P_{50} enhanced the influence of ϕ (Fig. 3B and C). Similar to normoxia, decreasing the P_{50} from 40 to 25 Torr in moderate hypoxia reduced the most beneficial Hill coefficient, from a value greater than 5 to 2.6 (Fig. 3D and E). Compared to normoxia, moderate hypoxia favoured more Hb-O_2 cooperativity (higher n values) at both P_{50} values.

Changing other physiological variables had similar effects in moderate hypoxia as in normoxia (Fig. 4B). The only substantial exception to this was the interaction between P_{50} on C_{Hb}, $\dot{Q}$, and $D_{T_{O_2}}$. The benefit of increasing C_{Hb} and $\dot{Q}$ was similar between normoxia and moderate hypoxia at a P_{50} of 40 Torr, but C_{Hb} and $\dot{Q}$ increased $\dot{V}_{O_2}$ by only 13% at a P_{50} of 25 Torr (compared to 20% in normoxia). The benefit of increasing $D_{T_{O_2}}$ at a P_{50} of 40 Torr was higher in moderate hypoxia (38%) than in normoxia (30%), but at a P_{50} of 25 Torr $D_{T_{O_2}}$ had nearly equivalent effects (56% and 57%).

3.5. Sensitivity analysis in severe hypoxia

The effects of Hb properties on oxygen consumption during exercise were very different in severe hypoxia than in normoxia and moderate hypoxia. The lower P_{50} still enhanced the influence of the Bohr effect, but the difference between the two P_{50} values was much greater (Fig. 3B and C). Altering the Bohr coefficient had less influence on $\dot{V}_{O_2}$ at a P_{50} of 40 Torr (much shallower slope), and increasing ϕ beyond about 0.6 did not increase $\dot{V}_{O_2}$. The interaction between P_{50} and the Hill coefficient was also different in severe hypoxia; lowering the P_{50} increased the most beneficial Hill coefficient from 1.5 to 2.4 (Fig. 3D and E). In normoxia and moderate hypoxia, lowering P_{50} reduced the most beneficial value for n. Furthermore, whereas moderate hypoxia favoured more Hb-O_2 cooperativity than normoxia (higher n) at both P_{50} values, severe hypoxia favoured less cooperativity than normoxia.

The effects of changing other physiological variables were also very different in severe hypoxia than in normoxia and moderate hypoxia (Fig. 4C). At a P_{50} of 40 Torr, the benefit of increasing C_{Hb}, $\dot{Q}$, and $\dot{V}$ increased (to 10%, 10%, and 41%, respectively), and the benefit of increasing $D_{T_{O_2}}$ decreased (to only 2%). The effects of lowering P_{50} on the benefit of C_{Hb} and $\dot{Q}$ were reversed in severe hypoxia compared to normoxia and moderate hypoxia, because the benefit of increasing them was much lower (only 2%) at a P_{50} of 25 Torr. Furthermore, the negative effect of the lower P_{50} on the influence of $\dot{V}$ was reduced in severe hypoxia (increasing $\dot{V}$ enhanced $\dot{V}_{O_2}$ by 33%). The strong interaction between P_{50} and $D_{T_{O_2}}$ was still apparent in severe hypoxia, because increasing $D_{T_{O_2}}$ at a P_{50} of 25 Torr enhanced $\dot{V}_{O_2}$ by 34%. Increasing $D_{L_{O_2}}$ still had a minor influence on $\dot{V}_{O_2}$ in severe hypoxia.

Changing the effect of temperature on Hb P_{50} had a large influence on $\dot{V}_{O_2}$, for starting P_{50} values of both 40 and 25 Torr, but its influence was greater at the lower P_{50} (Fig. 5). The significance of the temperature effect must be taken with some caution, however, as the degree of pulmonary blood cooling during flight in low ambient temperatures is unknown. This part of the model assumes that pulmonary blood is cooled 10 °C. If this does occur, then at a physiologically realistic value for the temperature effect (~1.5 Torr/°C; Maginniss et al., 1997) $\dot{V}_{O_2}$ could increase by 40–60%.

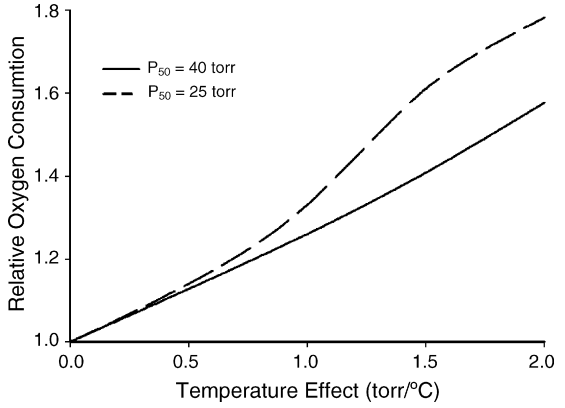

Fig. 5. The effect of varying the temperature effect on the P_{50} of haemoglobin (Hb) on oxygen consumption during exercise in severe hypoxia ($P_{I_{O_2}}$ of 30 Torr). Arterial blood was assumed to be cooled by 10 °C in the lungs (see Section 2). The effects of each variable were assessed at the P_{50} of pekin ducks (40 Torr; solid) as well as the P_{50} of bar-headed geese (25 Torr; dashed). Data were collected using the theoretical model of oxygen transport that is described in Section 2.

3.6. $\dot{V}$ and $D_{T_{O_2}}$ during flight at extreme altitude

The results of the sensitivity analysis suggest that ventilation rate and tissue diffusion capacity are likely to have the greatest influence on $\dot{V}_{O_2}$ in severe hypoxia. Using a simple set of equations, we sought to assess the possible combinations of physiological variables that could sustain the $\dot{V}_{O_2}$ required for flight at high altitude. Bar-headed geese have been known to fly at altitudes of at least 9000 m ($P_{I_{O_2}} \approx 38$ Torr), and during flight in a wind tunnel they consume oxygen at rates of 15–20 mmol/min (Ward et al., 2002). The possible combinations of $\dot{V}$ and $P_{E_{O_2}}$ that could sustain this $\dot{V}_{O_2}$ were calculated using Eq. (7) (Fig. 6A). The minimum ventilation rate for this $\dot{V}_{O_2}$ at a $P_{I_{O_2}}$ of 38 Torr is approximately 7 L/min, but this assumes complete removal of O_2 from the inspired gas. Over a physiologically realistic range of $P_{E_{O_2}}$, $\dot{V}$ would need to be between 10 and 20 L/min (depending on the amount of O_2 extracted from the inspired gas; see arrows in Fig. 6A), which is approximately two- to four-fold higher than the starting $\dot{V}_{O_2MAX}$ values from the in vivo pekin duck data used in the sensitivity analysis.

The possible combinations of $D_{T_{O_2}}$ and $\dot{Q}$ that could sustain $\dot{V}_{O_2}$ during flight were determined primarily using Eqs. (3) and (8) (Fig. 6B). The calculations were made with two different values for Pa_{O_2} (38 and 30 Torr), so the effect of a low versus high inspired-arterial P_{O_2} difference could be determined. By doing so, it was determined that when the inspired-arterial P_{O_2} difference was low ($Pa_{O_2} \approx 38$ Torr), concurrent two- to three-fold increases in $D_{T_{O_2}}$ (0.135 to ~0.4 mmol/Torr min) and $\dot{Q}$ (1.8 to ~6.0 L/min) above

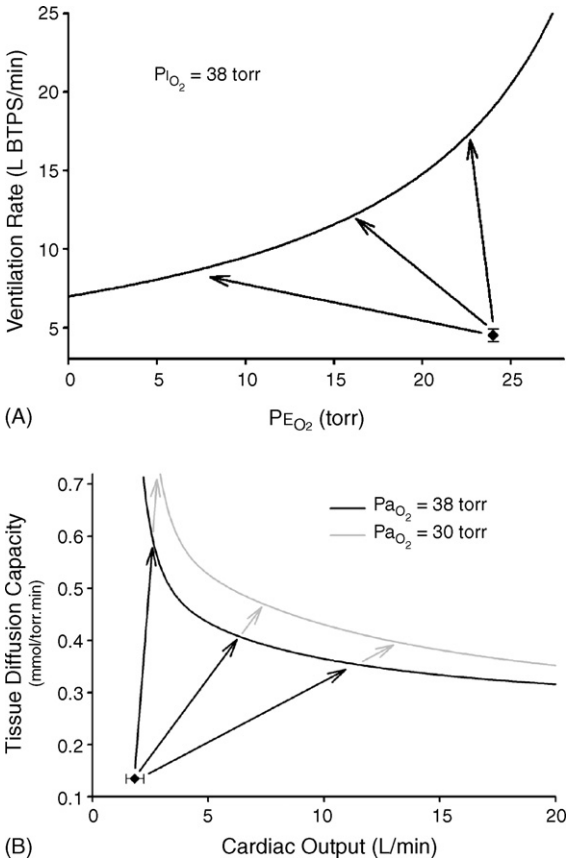

Fig. 6. A theoretical assessment of the ventilation rates (A) and tissue diffusion capacities (B) that could achieve the rate of oxygen consumption typical of an adult bar-headed goose during flight ($\dot{V}_{O_2} = 15$ mmol/ min). A $P_{I_{O_2}}$ of 38 Torr simulates an altitude of approximately 9000 m. In (A), the possible combinations of $\dot{V}$ and $P_{E_{O_2}}$ were calculated using Eq. (7). In (B), the possible combinations of $D_{T_{O_2}}$ and $\dot{Q}$ were determined with Eqs. (3)–(5) and (8), for two possible values for Pa_{O_2} (38 Torr in black, 30 Torr in grey). ♦ The condition for pekin ducks (data from Black and Tenney, 1980; see starting values in Table 2). As the arrows in (A) and (B) show, greater increases in either $\dot{V}$ or $D_{T_{O_2}}$ reduce the required lung oxygen extraction or cardiac output, respectively, to sustain the $\dot{V}_{O_2}$ during flight. P_{50} was set at 25 Torr, and the values for all other parameters are shown in Table 2.

the starting values from pekin ducks near $\dot{V}_{O_2MAX}$ (middle arrow, Fig. 6B) could achieve the required $\dot{V}_{O_2}$ for flight. Larger increases in either $D_{T_{O_2}}$ or $\dot{Q}$ would reduce the amount of change required in the other variable (see arrows, Fig. 6B). Furthermore, if the inspired-arterial P_{O_2} difference was higher, much greater increases in $D_{T_{O_2}}$ and $\dot{Q}$ would be required (grey curve, Fig. 6B). Overall, the assessment in Fig. 6 suggests that physiologically reasonable increases in $\dot{V}$, $D_{T_{O_2}}$, and $\dot{Q}$ (two- to three-fold) from the starting values obtained from pekin ducks near $\dot{V}_{O_2MAX}$ (Table 2) would allow species like the bar-headed goose to fly at extreme altitude.

4. Discussion

Unlike in vivo studies, theoretical sensitivity analyses allow individual physiological variables to be altered independently so their individual effects on oxygen consumption can be assessed. By applying this analysis to hypoxia in birds, we feel we can predict which factors most likely limit oxygen consumption and exercise performance (Table 1). As a consequence, our analysis identifies which steps in the oxygen cascade can provide the basis for adaptive change in birds that evolved for high altitude flight, namely ventilation and tissue diffusion capacity.

4.1. Assumptions and starting data of the model

As discussed in Section 2.3, the assumptions in our model (as outlined in Section 2) are similar to those in previous theoretical studies of oxygen transport and a thorough discussion of many of these assumptions are included in these earlier works (Wagner, 1996b; Wang and Hicks, 2002, 2004). While the assumptions of the model may in some cases limit its predictive power, they eliminate a great deal of complexity, much of which cannot be modelled due to a lack of mechanistic knowledge. The model assumes that ventilation/perfusion heterogeneity in the lungs and metabolism/perfusion heterogeneity in the tissues can be treated as part of $D_{L_{O_2}}$ and $D_{T_{O_2}}$. The effects of changing these 'apparent' lung and tissue diffusion capacities should therefore be considered to represent the role of lung and tissue gas exchange in general. Otherwise, an exceedingly complex multi-compartment heterogeneous model would be necessary, and in vivo data addressing these potential heterogeneities are lacking, particularly at the tissues (but see Powell and Wagner, 1982; Schmitt et al., 2002). With regard to $D_{T_{O_2}}$ the model also treats the 'tissues' as a single compartment. Whereas diffusion capacity of any one tissue is assumed to be constant at $\dot{V}_{O_2MAX}$, regardless of the level of hypoxaemia, different tissues may have different tissue diffusion capacities (Hogan et al., 1988). Indeed, the observation that the calculated starting values for $D_{T_{O_2}}$ in the sensitivity analysis increase progressively from normoxia to severe hypoxia suggests that blood flow is being redistributed as oxygen levels fall. Interestingly, in humans $D_{T_{O_2}}$ declines with altitude (Wagner, 1996b), suggesting that there are differences in muscle perfusion with altitude between birds and mammals, or differential responses of the structural determinants of tissue diffusion capacity. Unfortunately, as mentioned above, the assumptions in the model do not allow us to predict the potential adaptive benefits of these heterogeneities for high altitude performance.

Since our interest was in the factors limiting exercise performance at altitude, the starting data for our model were obtained from previous studies on pekin ducks near maximal oxygen consumption. These ducks were exercising on a treadmill, however, and were not flying. Unfortunately, to the best of our knowledge only one previous study has made all the required measurements for this analysis during flight, and this was only done in normoxia (in pigeons, Butler et al., 1977). Pekin ducks are the only species for which we could find all the required measurements for our analysis during exercise in both normoxia and hypoxia (Black and Tenney, 1980; Kiley et al., 1985). Only the lung and tissue diffusion capacities remained to be calculated in our analysis, but previous experimental determinations of $D_{L_{O_2}}$ in pekin ducks were similar to the values calculated in this study (Scheid et al., 1977). Similar values for $D_{T_{O_2}}$ are not available.

4.2. What limits exercise performance in birds?

As is the case in mammals, our analysis suggests that tissue diffusion capacity poses the greatest limitation to exercise performance in birds during normoxia (Wagner, 1996a,b). Qualitatively, this appears to be the case regardless of haemoglobin O_2 affinity, although

the benefit of increasing $D_{T_{O_2}}$ was greater at a low P_{50}. In normoxia, increasing $D_{T_{O_2}}$ may be more beneficial to birds with a lower P_{50} because their Hb does not desaturate until a lower P_{O_2}. Mean capillary P_{O_2} would therefore be lower, as would the driving force for diffusion into exercising muscle, so a higher $D_{T_{O_2}}$ should be required to maintain $\dot{V}_{O_2}$ during exercise. At this lower P_{50}, $\dot{Q}$ and C_{Hb} also appear to limit exercise performance in birds, but changes in $\dot{V}$ have less influence, as in mammals and some reptiles (Wagner, 1996b; Frappell et al., 2002). This was not the case at the higher P_{50}, where $D_{T_{O_2}}$ was the only substantial limitation to $\dot{V}_{O_2}$. Because this model ignores the contribution of changes in cardiac output to changes in perfusion of specific tissues and tissue blood volume, however, the influence of cardiac output may be greater than determined by our analysis. $D_{L_{O_2}}$ does not appear to pose much of a limitation to $\dot{V}_{O_2}$ in normoxia, as is the case in humans and some but not all lizards (Wagner, 1996b; Frappell et al., 2002; Wang and Hicks, 2004).

The physiological variables limiting exercise performance in birds during moderate hypoxia are similar to those limiting performance in normoxia. $D_{T_{O_2}}$ continues to pose the greatest limitation, and limitations imposed by the circulation ($\dot{Q}$ and C_{Hb}) are still greater at a lower P_{50}. Unlike normoxia, however, $\dot{V}_{O_2}$ in moderate hypoxia appears to be limited less by the circulation and more by respiratory variables, as is also the case in humans (Wagner, 1996b).

The most substantial difference between severe hypoxia and normoxia/moderate hypoxia is in the effects of altering ventilation. Ventilation appears to become a major limitation to exercise performance at extreme altitude. $D_{T_{O_2}}$ also appears to limit $\dot{V}_{O_2}$ in severe hypoxia, but only at lower P_{50} values. This is not entirely unsurprising: in severe hypoxia the venous blood of pekin ducks (a species which has a higher P_{50}) is almost completely deoxygenated in vivo (Black and Tenney, 1980), so there are no possible benefits of increasing $D_{T_{O_2}}$. At the lower P_{50}, there is a substantially higher arterial oxygen content, so more oxygen can be removed, and increasing $D_{T_{O_2}}$ can have a greater influence. In humans during severe hypoxia, $D_{T_{O_2}}$, $D_{L_{O_2}}$, and $\dot{V}$ have the greatest influence on exercise performance (Wagner, 1996b). That $D_{L_{O_2}}$ appears to pose less of a limitation to exercise performance at extreme altitude in birds may reflect the extremely small diffusion distances across the lungs of most birds (Maina and King, 1982; Powell and Mazzone, 1983; Shams and Scheid, 1989). However, the apparent $D_{L_{O_2}}$ may decrease and become limiting during high intensity exercise at altitude if lung edema occurs due to pulmonary vasoconstriction. In this regard, it is interesting that pulmonary blood pressure does not rise in bar-headed geese during hypoxaemia, in contrast to many other birds and mammals (Faraci et al., 1984a).

4.3. Potential adaptations for high altitude flight in birds

Hb-O_2 affinity is known to be higher in many species adapted to high altitude, so it is perhaps not surprising that our modelling suggests that this trait is adaptive in high altitude birds. Historically, a high Hb-O_2 affinity was believed to be beneficial because of the effect of a low P_{50} on oxygen loading at the lungs (Samaja et al., 2003). The benefit of a low P_{50} alone on total oxygen consumption is unclear, however, because Hb with a high affinity inhibits oxygen unloading at the tissues. This is perhaps why decreasing P_{50} alone could only increase $\dot{V}_{O_2}$ during exercise by 10–20% in this study. It has been suggested that other factors influencing Hb-O_2 affinity, such as the Bohr effect, could concurrently facilitate oxygen unloading; however, our results suggest that a larger Bohr effect has surprisingly little influence on $\dot{V}_{O_2MAX}$. Consistent with this, the Bohr effect is actually lower in the high altitude bar-headed goose than in other waterfowl (Liang et al., 2001).

Tissue diffusion capacity should also be adaptive in high altitude birds with a high haemoglobin O_2 affinity. In the present study, a simultaneous decrease in P_{50} (from 40 to 25 Torr) and increase in $D_{T_{O_2}}$ (two-fold) increased $\dot{V}_{O_2}$ by 51%. Thus, in high flying birds that are known to have a low P_{50}, such as the bar-headed goose and Rüppell's griffon (*Gyps rueppellii*), increases in flight muscle diffusion capacity should be of extreme importance. This suggestion is supported by research demonstrating greater muscle capillarization in bar-headed geese than in low altitude fliers (Snyder et al., 1984; Fedde et al., 1985), as the size of the capillary–muscle fibre interface is known to be the primary structural determinant of O_2 flux into the muscle (Hepple, 2000; Mathieu-Costello, 2001). Increased

$D_{T_{O_2}}$ in bar-headed geese may be an adaptation specific to high altitude, but could also be a consequence of other factors (e.g., lower mean capillary P_{O_2} in normoxia) as discussed in Section 4.2. Regardless of the evolutionary forces producing the change, increased $D_{T_{O_2}}$ should be tremendously beneficial to high altitude fliers with a low P_{50}. Furthermore, our results suggest that there may be interesting interactions between P_{50} and $D_{T_{O_2}}$ in general, which should be considered when assessing the adaptive benefit of a high Hb-O_2 affinity in high altitude animals.

Our analysis suggests that an enhanced capacity to increase ventilation should also benefit birds significantly in severe hypoxia, and could therefore be an important source of adaptation for high altitude flight. This is likely true regardless of P_{50}; although there is a small amount of interaction between P_{50} and ventilation, increasing $\dot{V}$ always had a substantial effect on oxygen consumption. Data from the literature addressing this possibility have unfortunately been inconclusive. Both bar-headed geese and pekin ducks can effectively increase ventilation, thus reducing the inspired-arterial O_2 difference, during severe poikilocapnic hypoxia at rest, as well as during moderate poikilocapnic hypoxia and running exercise (Black and Tenney, 1980; Kiley et al., 1985; Fedde et al., 1989; Shams and Scheid, 1989). However, differences between the capacities of the respiratory systems of these species may only become apparent when ventilatory demands are increased to the levels seen during flight. Furthermore, because hyperventilation during flight at altitude should cause a severe respiratory hypocapnia/alkalosis, high altitude birds may benefit from a lower CO_2/pH sensitivity of ventilation. Lower CO_2/pH sensitivity would enhance the poikilocapnic hypoxic ventilatory response (HVR), making it more similar to an isocapnic HVR. The difference between poikilocapnic and isocapnic HVRs (i.e., the CO_2/pH sensitivity of ventilation) in low and high altitude birds have not been compared (but see Powell et al., 2000), and thus it remains to be determined whether a greater capacity to increase ventilation is an important adaptation for high altitude flight.

Differences in the Bohr effect or the Hill coefficient did not appear to be adaptive for flight at high altitude. Neither variable had a major influence on oxygen consumption in severe hypoxia, as the benefits of changing these variables did not generally exceed 10%. Previous studies in many bird species have shown that the Hill coefficient increases with P_{O_2}, possibly enhancing oxygen transport (Maginniss et al., 1997), but this possibility was not addressed in the present analysis.

In contrast to the Bohr effect and Hill coefficient, the temperature effect on Hb-O_2 binding affinity may have a substantial effect on oxygen consumption, and may therefore be a source of adaptive change for high altitude flight (Maginniss et al., 1997). An effect of temperature on $\dot{V}_{O_2}$ may arise if hyperventilation during flight at extreme altitude cools the pulmonary blood. This would reduce the P_{50} of Hb in the lungs, and thus facilitate oxygen uptake. When this blood enters the exercising muscles it would then be rewarmed to body temperature, and oxygen would be released from Hb. Our modelling suggests that a temperature effect on Hb could significantly enhance $\dot{V}_{O_2}$. The greater the difference in temperature between blood in the lungs and in the muscles, and the greater the temperature effect on Hb-O_2 binding, the greater the increase in $\dot{V}_{O_2}$. At normal levels of temperature sensitivity, the increase in $\dot{V}_{O_2}$ was approximately 5% for every 1 °C difference. Altering the magnitude of the temperature effect on Hb while allowing lung temperature to fall could therefore be adaptive at high altitude. At present, however, it is unknown whether the Hb of high altitude birds has a heightened sensitivity to temperature, or whether pulmonary blood is actually cooled during high altitude flight.

Increasing other physiological variables that we studied, namely maximum cardiac output, Hb concentration, and lung diffusion capacity, are likely of little selective advantage during adaptation to high altitude. As discussed above, these traits do not seem to limit exercise performance at extreme altitude, and theoretically increasing them had only small benefits for oxygen transport. The effect of some other variables (e.g., blood flow distribution, pH regulation, tissue oxidative capacity, etc.; Table 1) could not be examined using our model, so we cannot exclude an adaptive benefit of these physiological traits.

4.4. Conclusions

Using a theoretical sensitivity analysis that allows individual physiological variables to be altered independently, we have identified the factors most likely to limit oxygen consumption and exercise performance in

birds, and by extension, the physiological changes that are likely adaptive for high altitude flight. The adaptive benefits of some of these changes, in particular haemoglobin oxygen affinity, are already well established for high flying birds. For other traits, such as an enhanced hypoxic ventilatory response or O_2 diffusion capacity of flight muscle, adaptive differences have not been conclusively recognized in studies in vivo. Furthermore, the beneficial interaction between increasing $D_{T_{O_2}}$ and decreasing haemoglobin P_{50} has not yet been demonstrated in vivo. Our theoretical analysis suggests that changes in these respiratory processes could also adapt birds to environmental extremes, and future studies should explore these findings.

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