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Altitude acclimatization, hemoglobin-oxygen affinity, and circulatory oxygen transport in hypoxia

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ABSTRACT

In mammals and other air-breathing vertebrates that live at high altitude, adjustments in convective O_2 transport via changes in blood hemoglobin (Hb) content and/or Hb-O_2 affinity can potentially mitigate the effects of arterial hypoxemia. However, there are conflicting views about the optimal values of such traits in hypoxia, partly due to the intriguing observation that hypoxia-induced acclimatization responses in humans and other predominantly lowland mammals are frequently not aligned in the same direction as evolved phenotypic changes in high-altitude natives. Here we review relevant theoretical and empirical results and we highlight experimental studies of rodents and humans that provide insights into the combination of hematological changes that help attenuate the decline in aerobic performance in hypoxia. For a given severity of hypoxia, experimental results suggest that optimal values for hematological traits are conditional on the states of other interrelated phenotypes that govern different steps in the O_2-transport pathway.

1. Introduction

When humans ascend to high-altitude, the initial acclimatization response to environmental hypoxia involves a consistent suite of hematological changes, including an increase in hemoglobin (Hb) concentration and a reduction in Hb-O_2 affinity (Monge and Leon-Velarde, 1991; Storz et al., 2010b; Ivy and Scott, 2015; Storz and Scott, 2019). The hypoxia-induced increase in [Hb] initially stems from a reduction in plasma volume (hemoconcentration) and – after a period of weeks – increased red blood cell production, which is sustained by renal synthesis and release of erythropoietin. The hypoxia-induced reduction in Hb-O_2 affinity stems from changes in red cell metabolism that increase the intraerythrocytic concentration of 2,3-diphosphoglycerate (DPG [also referred to as biphosphateglycerate]), an allosteric cofactor with inhibitory effects on Hb-O_2 binding. Many previous workers assumed that such responses must be physiologically beneficial in hypoxia (Aste-Salazar and Hurtado, 1944; Lenfant et al., 1968, 1969, 1971; Eaton et al., 1969; Frisancho, 1975), in keeping with the intuitive expectation that acclimatization responses to environmental stimuli are generally well-matched to the range of conditions experienced by a given species.

There are sound reasons to expect that the above-mentioned hematological changes could be physiologically beneficial under conditions of environmental hypoxia. An increased [Hb] increases arterial O_2 content (C_aO_2) for a given O_2 partial pressure (PO_2), thereby augmenting blood O_2 conductance if cardiac output remains constant. Likewise, a reduced Hb-O_2 affinity promotes O_2-unloading to metabolizing tissues, which could conceivably compensate for suboptimal O_2 delivery (Lenfant et al., 1971). Intriguingly, however, the typical acclimatization responses to hypoxia in species like humans that have predominantly lowland ancestries are frequently not aligned in the same direction as evolved phenotypic changes in species that are long-term highland natives. For example, high-altitude species that are manifestly well-adapted to chronic hypoxia do not generally have highly elevated [Hb] and instead tend to exhibit values similar to those observed in lowland relatives living at or near sea level (Storz et al., 2010b; Storz and Scott 2019). Likewise, in contrast to the hypoxia-induced reduction in Hb-O_2 affinity that is typical of humans and other lowland mammals, many high-altitude vertebrates have evolved genetically based increases in Hb-O_2 affinity in comparison with lowland relatives (Natarajan et al., 2015b, 2016, 2018; Storz, 2016, 2019; Jendroszek et al., 2018; Zhu et al., 2018; Signore et al., 2019; Signore and Storz, 2020). How can we reconcile these contrasting patterns of acclimatization and evolutionary change in lowlanders and highlanders?

There is a growing appreciation that acclimatization responses to environmental hypoxia are a fallible guide for inferring adaptive value

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(Storz et al., 2010b; Dempsey and Morgan, 2015). Indeed, several aspects of hypoxia adaptation in highland natives appear to involve genetically based changes that counteract or compensate nonadaptive acclimatization responses (Storz et al., 2010b; Storz and Scott, 2019, 2021). Another possibility is that the adaptive value of changes in any one trait may be conditional on physiological context and patterns of phenotypic integration, and may therefore differ in species with lowland and highland ancestries. For example, a particular hematological response to hypoxia may be beneficial in the context of a native highlander physiology that is characterized by evolved changes in numerous, interrelated traits, whereas a qualitatively distinct acclimatization response may be more beneficial in the context of a native lowland physiology.

Here we review theoretical and experimental results that suggest hypotheses about the adaptive significance of hypoxia-induced changes in hematological traits. We highlight case studies involving rodents and humans that provide insights into the combination of hematological changes that help attenuate the expected decline in aerobic performance in hypoxia.

2. The O_2-transport pathway and the challenge of environmental hypoxia

In animals that rely on aerobic metabolism to meet their energy requirements, sustained ATP synthesis requires the O_2-transport system to match O_2 supply (delivery of O_2 to tissue mitochondria) and metabolic demand (chemical utilization of O_2 by oxidative phosphorylation in the mitochondrial electron transport chain). This balance is maintained by ventilatory and cardiovascular adjustments that jointly ensure an uninterrupted supply of O_2 to the cells of metabolizing tissues. The transport of O_2 from atmospheric air to cellular mitochondria involves four serially integrated transfer steps: (i) ventilation of the lungs; (ii) diffusion of O_2 across the alveolar-capillary barrier (i.e., from inspired air into the blood); (iii) circulatory transport of O_2 in the blood; and (iv) diffusion of O_2 from capillary blood to cellular mitochondria, where it is then used as the terminal electron acceptor in aerobic respiration. Changes in PO_2 across these alternating convective and diffusive steps provide the driving force for O_2 transport and account for the overall pressure gradient between the PO_2 of inspired air and the PO_2 in the mitochondria.

At high altitude, the reduced barometric pressure diminishes the O_2 pressure head of inspired air. Thus, to ensure an adequate O_2 flux to metabolizing tissues, physiological adjustments are required to minimize the inevitable decrement in PO_2 across successive transfer steps of the O_2-transport pathway (Bouverot, 1985; Scott and Milsom, 2006; Ivy and Scott, 2015; McClelland and Scott, 2019; Storz and Scott, 2019).

2.1. Convective O_2 transport by the cardiovascular system

As a description of convective O_2 transport by the cardiovascular system, the Fick Principle states that the rate of O_2 consumption by perfused tissue is equal to the product of blood flow to the tissue and the difference in O_2 content between the arterial blood entering the tissue capillary bed and the venous blood leaving the capillary bed. Accordingly, the rate of O_2 consumption ($\dot{V}O_2$) is the product of cardiac output (Q) and the difference in the O_2 contents of arterial and venous blood (C_aO_2 and C_vO_2, respectively):

$$\dot{V}O_2 = Q \times \left(C_aO_2 - C_vO_2\right) \quad (1.1)$$

This equation can also be written in terms of PO_2 and the blood-O_2 capacitance coefficient (βbO_2), which quantifies the amount of O_2 that is unloaded for a given arterial-venous difference in PO_2:

$$\dot{V}O_2 = Q \times \beta bO_2 \times \left(P_aO_2 - P_vO_2\right), \quad (1.2)$$

Where $\beta bO_2 = (C_aO_2 - C_vO_2)/(P_aO_2 - P_vO_2)$, the arterial-venous difference in O_2 content divided by the arterial-venous difference in PO_2.

The blood-O_2 capacitance coefficient, βbO_2, is determined by both blood Hb content and the O_2-binding properties of Hb (O_2-affinity and cooperativity). Whereas the O_2 capacitance of air or water is constant for a given temperature, the O_2 capacitance of the blood changes as a function of PO_2 due to the cooperativity of Hb-O_2 binding (ligation of a given heme in deoxyHb increases O_2-affinities of the remaining unliganded hemes and, conversely, ligand release by a given heme in oxyHb reduces O_2 affinities of the remaining liganded hemes). Consequently, Hb has a high O_2 affinity in the pulmonary capillaries where PO_2 is high, and a reduced affinity in the systemic capillaries where PO_2 is much lower.

2.2. Changes in convective O_2 transport are mediated by changes in the shape and position of the O_2-equilibrium curve

The O_2-equilibrium curve provides a way of graphically depicting the Fick principle when the vertical axis is expressed in terms of blood O_2 content. As illustrated in Fig. 1, the blood O_2 capacitance coefficient, βbO_2, is defined as the slope of the line joining the arterial and venous points on the sigmoidal curve. An increase in the slope of the line increases the arterial-venous difference in O_2 content ($C_aO_2 - C_vO_2$) for a given arterial-venous difference in O_2 tension ($P_aO_2 - P_vO_2$). Thus, increasing βbO_2 (via changes in Hb concentration and/or changes in Hb-O_2 affinity) increases the quantity of O_2 transported to the tissue for a given difference in PO_2 between the sites of O_2 loading and unloading.

2.3. Hypoxia-induced changes in [Hb] and its physiological significance

When the reduced PO_2 of inspired air results in a corresponding reduction in the PO_2 of arterial blood (P_aO_2), the decrement in blood O_2 conductance can be minimized by increasing cardiac output (Q) and/or βbO_2 (Fig. 1). All else being equal, an increase in blood [Hb] augments blood O_2 conductance by increasing C_aO_2, which can contribute to enhanced aerobic performance under conditions of environmental hypoxia as well as exercise-induced tissue hypoxia (Kanstrup and Ekblom, 1984; Ekblom and Berglund, 1991; Mairbaurl, 1994; Samaja et al., 2003; Calbet et al., 2006; Saunders et al., 2013). However, an excessively high [Hb] (polycythemia) can be counterproductive because the associated increase in blood viscosity compromises cardiac output, thereby offsetting the benefit of the elevated C_aO_2 for tissue O_2 delivery. The nonlinear relationship between hematocrit and aerobic exercise performance is well-documented in mice under normoxic conditions (Schuler et al., 2010). Although similar performance curves have not been measured in hypoxia, modeling results predict that the optimal hematocrit for exercise in hypoxia is not too different from that at sea-level, but there are more complex interaction effects with red cell [DPG] and deviations from the optimal hematocrit in either direction are predicted to more strongly curtail $\dot{V}O_{2max}$ (i.e., the phenotypic optimum is more sharply defined in hypoxia than in normoxia)(Winslow, 1988). Experimental data for rodents and humans indicate that the effect of changing hematocrit or blood [Hb] is highly dependent on changes in other cardiovascular traits during acclimatization (Gonzalez and Kuwahira, 2018).

Experiments on rats that involved isovolumic exchange transfusions revealed that changes in hematocrit had very different effects on exercise performance in acclimated and nonacclimated animals (Gonzalez et al., 1994). These experiments measured the maximum rate of O_2 consumption ($\dot{V}O_{2max}$), an integrated measure of aerobic performance that reflects the overall flux capacity of the O_2-transport system (McClelland and Scott, 2019; Scott and Dalziel, 2021). Increasing hematocrit in nonacclimated rats from ~45% to 60% attenuated the expected decline in hypoxic $\dot{V}O_{2max}$ by increasing C_aO_2 and the arterial-venous difference in O_2 content, changes which more than offset

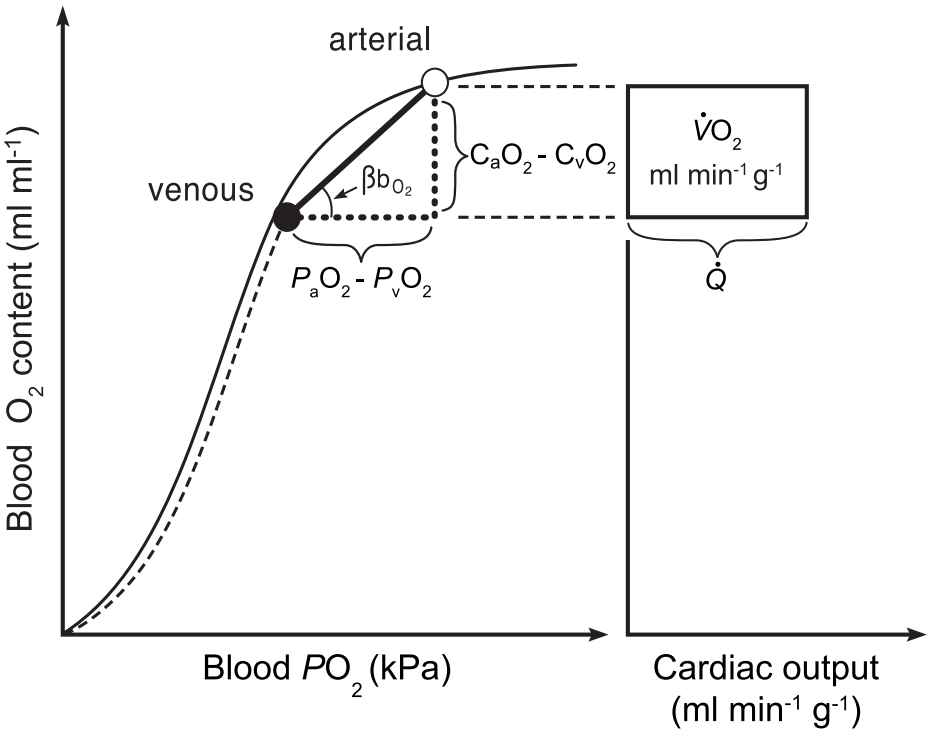

Fig. 1. Schematic illustration of blood O_2 transport. (A) O_2-equilibrium curve under the physicochemical conditions prevailing in arterial blood (a, solid curve, open symbol) and venous blood (v, dashed curve, closed symbol). The curve is a plot of blood O_2 content (y-axis) versus PO_2 (x-axis), with paired values for arterial and venous blood connected by a solid line. C_aO_2 - C_vO_2 denotes the arterial–venous difference in blood O_2 content, P_aO_2 - P_vO_2 denotes the corresponding difference in PO_2, βbO_2 denotes the blood O_2 capacitance coefficient (see text for details), Q denotes cardiac output, and $\dot{V}O_2$ denotes the rate of O_2 consumption. On the right-hand side of the graph, the area of the rectangle is proportional to total O_2 consumption, which can be enhanced by increasing Q and/or by increasing βbO_2. Increases in βbO_2 produce a corresponding increase in C_aO_2 - C_vO_2 through shifts in the shape or position of the O_2-equilibrium curve.

the associated decrease in cardiac output. By contrast, reducing hematocrit in acclimated rats from ~60% to 45% increased cardiac output but had no detectable effect on $\dot{V}O_{2max}$. The inverse relationship between hematocrit and cardiac output is not explained by changes in blood viscosity, and may instead reflect other changes that increase systemic vascular resistance (Gonzalez et al., 1994; Gonzalez and Kuwahira, 2018). Studies in humans have also demonstrated that hypoxic $\dot{V}O_{2max}$ is unaffected by acute, isovolumic reductions of [Hb] in polycythemic highlanders (Tufts et al., 1985; Winslow et al., 1985) and in altitude-acclimatized lowlanders (Sarnquist et al., 1986; Schaffartzik et al., 1993; Calbet et al., 2002, 2003). It appears that the increase in C_aO_2 associated with hypoxia-induced polycythemia is generally offset by a corresponding reduction in maximal cardiac output and a reduction in the fraction of cardiac output that is directed to contracting skeletal muscle at maximal exercise (Calbet et al., 2006; Gonzalez and Kuwahira, 2018). Although studies of acclimatized highland Tibetans and lowland Han Chinese subjects at 4200 m revealed that natural variation in [Hb] is negatively associated with $\dot{V}O_{2max}$ (Simonson et al., 2015), there is no direct evidence to suggest that the observed association reflects a causative relationship. In subjects that had lower $\dot{V}O_{2max}$ due to hypoxia-related deficiencies in convective O_2 transport, an elevated [Hb] may simply represent a secondary response to renal tissue hypoxia (the stimulus for erythropoietin production). Also, since Tibetan highlanders maintain elevated blood volumes in conjunction with non-elevated [Hb] in hypoxia (Stembridge et al., 2019), it seems likely that measures of total circulating Hb mass would be positively correlated with $\dot{V}O_{2max}$, as is generally the case for exercise performance in normoxia (Mairbaurl, 2013). Since measurements of blood [Hb] in g dL^{-1} are affected by plasma volume (and therefore only measure blood-O_2 carrying capacity per unit volume of cardiac output), measurements of total Hb mass provide a more relevant index of blood-O_2 carrying capacity when investigating determinants of $\dot{V}O_{2max}$.

In summary, an elevated [Hb] does not generally make a significant contribution to hypoxic $\dot{V}O_{2max}$ in rats or humans that are already acclimated/acclimatized to hypoxia (Calbet et al., 2006; Gonzalez and Kuwahira, 2018). At face value, this seems consistent with the general absence of polycythemia in longterm highland natives. Given that [Hb] is a hypoxia-responsive trait that reflects overall levels of tissue O_2 delivery (specifically, O_2-delivery to the kidney, which regulates the synthesis and release of erythropoietin in an O_2-dependent manner), the absence of polycythemia in highland natives may simply reflect the robustness of the O_2-transport pathway to a reduction in inspired PO_2. In other words, highland species may typically possess non-elevated [Hb] because they have evolved compensatory mechanisms to sustain adequate levels of tissue O_2 delivery in spite of low inspired PO_2, thereby dampening the hypoxic stimulus that induces the erythropoietin-mediated increase in [Hb] (Storz, 2021; Storz and Cheviron, 2021).

2.4. Optimal P_{50} in hypoxia

Adjustments in Hb-O_2 affinity provide an alternative means of increasing βbO_2 by shifting the steepest portion of the O_2-equilibrium curve within the operational range of the *in vivo* PO_2's at the sites of O_2 loading and unloading (Bouverot, 1985; Samaja et al., 2003). Although reduced Hb-O_2 affinity promotes O_2 unloading in the systemic circulation, it can jeopardize arterial O_2 saturation (S_aO_2) under conditions of environmental hypoxia. High-altitude animals therefore face the challenge of optimizing the trade-off between O_2 loading at the respiratory surfaces (which contributes to convective O_2 transport) and O_2 unloading in the systemic capillaries (which contributes to tissue O_2 extraction). The relative magnitudes of the opposing changes in O_2 loading and O_2 unloading determine the effect of shifting the O_2-equilibrium curve on $\dot{V}O_{2max}$, and this effect is expected to vary as a function of inspired PO_2.

For a given degree of hypoxia, is it preferable to have a left-shifted curve, a right-shifted curve, or neither? The obvious answer to this question is that it is preferable to have a left-shifted curve in the pulmonary capillaries and a right-shifted curve in the peripheral circulation. In fact, this is what actually happens due to differences in pH, CO_2, and temperature between the lungs and capillaries of working muscle. Although operating conditions for Hb change during circulatory transit

of red blood cells, intrinsic oxygenation properties of the protein remain unaltered, and such properties determine the set point for the modulation of O_2-affinity by temperature and allosteric effectors. Thus, insights into how the optimal blood P_{50} varies as a function of inspired PO_2 is key to understanding the possible adaptive significance of evolved changes in Hb-O_2 affinity in highland taxa.

The graphical framework shown in Fig. 2 reveals how shifts in the O_2-equilibrium curve can be expected to affect tissue O_2 delivery in hypoxia. We can see that under normoxia ($P_aO_2 = 10.7$ kPa [80 torr]), a reduced Hb-O_2 affinity (right-shifted O_2-equilibrium curve) results in a larger βbO_2 than an increased Hb-O_2 affinity (left-shifted O_2-equilibrium curve)(Fig. 2A). That is, it produces a larger increase in the slope of the line connecting the arterial and venous points on the curve, thereby maximizing tissue O_2 delivery (C_aO_2 - C_vO_2) for a given difference in PO_2 between the sites of O_2 loading and unloading (P_aO_2 - P_vO_2). By contrast, under hypoxia ($P_aO_2 = 3.6$ kPa [27 torr]), an increased Hb-O_2 affinity (left-shifted curve) produces the largest increase in βbO_2 because the arterial-venous difference in PO_2 spans a steeper portion of the curve (Fig. 2B). Fig. 2B also shows that a left-shifted curve preserves a higher P_vO_2 in hypoxia (Woodson 1988). The higher mean capillary PO_2 maintains a steeper pressure gradient for O_2 diffusion to the cells of working muscle.

The above considerations are consistent with theoretical and experimental results which suggest that a reduced Hb-O_2 affinity is generally beneficial for tissue O_2 delivery during exercise in normoxia or moderate hypoxia, whereas an increased Hb-O_2 affinity is beneficial in severe hypoxia (Turek et al., 1973, 1978; West and Wagner, 1980; Bencowitz et al., 1982; Willford et al., 1982; Samaja et al., 1986, 2003; Scott and Milsom, 2006). However, other theoretical results suggest that $\dot{V}O_{2max}$ is relatively insensitive to changes in blood P_{50} over a broad range of prevailing PO_2's (Wagner 1996a; b, 1997). Since changes in Hb-O_2 affinity might affect interactions with traits that govern different steps in the O_2-transport pathway, it is difficult to predict the effect of such changes on systematic O_2 transport in the intact organism (Wearing and Scott, 2021).

2.5. *Hypoxia-induced changes in Hb-O_2 affinity: effects of red cell DPG concentration and acid-base balance*

In humans and other mammals, the hypoxia-induced reduction in Hb-O_2 affinity (as measured by the 'standard P_{50}', pH 7.40, $PCO_2 = 40$ torr, 37°) is largely attributable to an increase in the red cell concentration of the allosteric effector DPG, which is a metabolite of glycolysis. The increase in red cell DPG concentration mainly stems from a hypoxia-induced increase in ventilation. The resultant respiratory alkalosis stimulates red cell glycolytic activity which, in turn, increases DPG synthesis (Rapoport et al., 1977). Since DPG preferentially binds and stabilizes deoxyHb, an increase in the relative concentration of Hb liganded with DPG reduces Hb-O_2 affinity by shifting the allosteric equilibrium in favor of the low-affinity (deoxy) T-state (Torrance et al., 1970/71; Lenfant et al., 1971; Mairbaurl et al., 1986; Mairbaurl et al., 1993; Mairbaurl, 1994). The hypoxia-induced stimulation of erythropoiesis can also reduce Hb-O_2 affinity by producing a downward shift in the mean age of circulating red blood cells, as newly produced red cells have higher [DPG] than older cells (Mairbaurl et al., 1990; Mairbaurl, 1994; Samaja et al., 2003). Hypoxia-induced respiratory alkalosis has offsetting effects on Hb-O_2 affinity *in vivo*: the increase in red cell [DPG] allosterically reduces Hb-O_2 affinity, but – depending on the severity of hypoxia – this may be counterbalanced by the increase in red cell pH which promotes an increased affinity via the Bohr effect (Winslow et al., 1984; Mairbaurl et al., 1990; Samaja et al., 1997, 2003). In a study conducted at Rifugio Regina Margherita, located on the summit of Punta Gnifetti (4559 m) in the Pennine Alps, *in vivo* O_2-equilibrium curves were measured in climbers over a 5-day acclimatization period. Results revealed that the affinity-reducing effect of increased red cell [DPG] was offset by the affinity-increasing effect of respiratory alkalosis, such that *in vivo* blood P_{50} in acclimatized subjects was unchanged from the pre-acclimatization value (Mairbaurl et al., 1990). During the 1981 American Medical Research Expedition to Everest, blood gases and in vivo O_2-equilibrium curves were measured in climbers over the course of several weeks at different stages of the ascent and subsequent descent. At elevations above ~6000 m, the affinity-increasing effect of respiratory alkalosis more than counteracted the DPG effect. On the summit (8848 m), extreme P_aCO_2 (1.00 kPa [= 7.5 torr]) and arterial pH (>7.7) yielded an estimated *in vivo* blood P_{50} of 2.59 kPa (=19.4 torr) (Winslow et al., 1984). According to Winslow (2007:124), "Without this striking increase in O_2 affinity, it is likely that Everest could not be climbed without supplemental O_2".

The general direction of evolved (genetically based) changes in Hb-O_2 affinity in high-altitude vertebrates runs counter to the DPG-mediated acclimatization effect (which right-shifts the O_2-equilibrium curve) and is more consistent with the alkalosis-mediated effect (which left-shifts the curve) (Storz, 2016, 2019). There is a surprisingly strong, positive relationship between Hb-O_2 affinity and native elevation in birds (based on *in vitro* measurements of purified Hb), but the pattern is far more equivocal in mammals. In any mammalian group for which sufficient data exist, it is not difficult to find exceptions. Among carnivores, for example, Tibetan wolves (*Canis lupus laniger*) have evolved an increased Hb-O_2 affinity and enhanced Bohr effect relative to lowland canids (Signore et al., 2019), but snow leopards (*Panthera uncia*) have Hb oxygenation properties indistinguishable from those of house cats (Janecka et al., 2015). Likewise, among rodents, there are some alpine and subalpine taxa that have evolved increased Hb-O_2 affinities in comparison with lowland relatives (Storz et al., 2010a) and other taxa

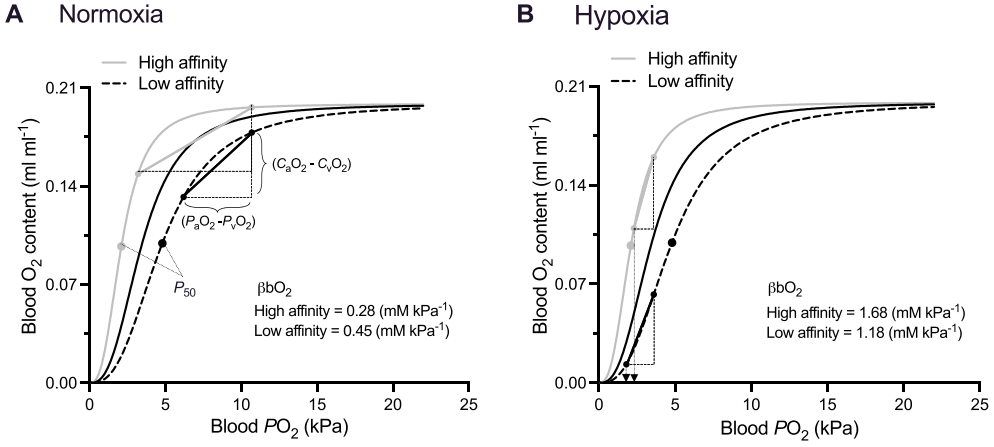

Fig. 2. O_2-equilibrium curves showing the effect of changes in Hb-O_2 affinity on tissue O_2 delivery under conditions of normoxia (A) and hypoxia (B). In panel B, the PO_2 for venous blood (P_vO_2) is marked by a vertical grey line that extends to the x-axis. P_{50}, the PO_2 at which Hb is 50% saturated. Each change in Hb-O_2 affinity produces a shift in P_vO_2, but the PO_2 of arterial blood (P_aO_2) is assumed to remain constant. Note that in normoxia (A) the right-shifted curve maximizes βbO_2 and preserves a higher P_vO_2 (an overall index of tissue oxygenation). In hypoxia, by contrast, the left-shifted curve maximizes βbO_2 and preserves a higher P_vO_2 relative to the right-shifted curve.

that do not conform to any consistent altitudinal trend (Revsbech et al., 2013). Comparative data can provide a rich source of adaptive hypotheses that can be tested with manipulative experiments, but it is important to recognize that most evolutionary trends are not completely uniform and consistent. Also, measurements of standard P_{50}'s for purified Hbs are informative about evolved changes in protein function, but it is also of interest to know to what extent such measurements reflect *in vivo* blood P_{50}'s under natural conditions.

3. Empirical case studies

The most direct means of assessing whether the optimal Hb-O_2 affinity varies in relation to ambient PO_2 is to measure performance curves that relate $\dot{V}O_{2max}$ to blood P_{50} under normoxia and varying degrees of hypoxia. The case studies described below provide insights into how changes in blood P_{50} affect physiological performance in hypoxia, and suggest hypotheses about the conditions in which changes in one direction or the other may be adaptive.

3.1. Exercise performance in rats with pharmacologically altered Hb-O_2 affinity

McCanse et al. (1999) and Henderson et al. (2000) used a combination of sodium cyanate treatments and blood transfusions in rats to assess the effect of increasing Hb-O_2 affinity on exercise performance in normoxia and varying degrees of hypoxia. Cyanate increases Hb-O_2 affinity by irreversibly carbamylating the amino termini of the α- and β-chains of the $\alpha_2\beta_2$ Hb tetramer. In normoxia, cyanate-treated rats had lower $\dot{V}O_{2max}$ than controls, which was clearly attributable to a lower tissue O_2 extraction (Fig. 3A). A comparison of the O_2-equilibrium curves in Fig. 3A indicates that the P_vO_2 of the 'high-affinity' cyanate-treated rats was considerably lower than that of the controls, reflecting a diminished PO_2 diffusion gradient between capillary blood and muscle cells. In severe hypoxia, by contrast, the difference in $\dot{V}O_{2max}$ between the treatment and control groups all but disappeared (Fig. 3B). This is explained by the fact that the increased Hb-O_2 affinity in cyanate-treated rats helped safeguard S_aO_2, thereby minimizing the drop in C_aO_2 in spite of the dramatically reduced P_aO_2. Consequently, the increased rate of convective O_2 transport compensated for the corresponding drop in tissue O_2 extraction. At even more severe levels of

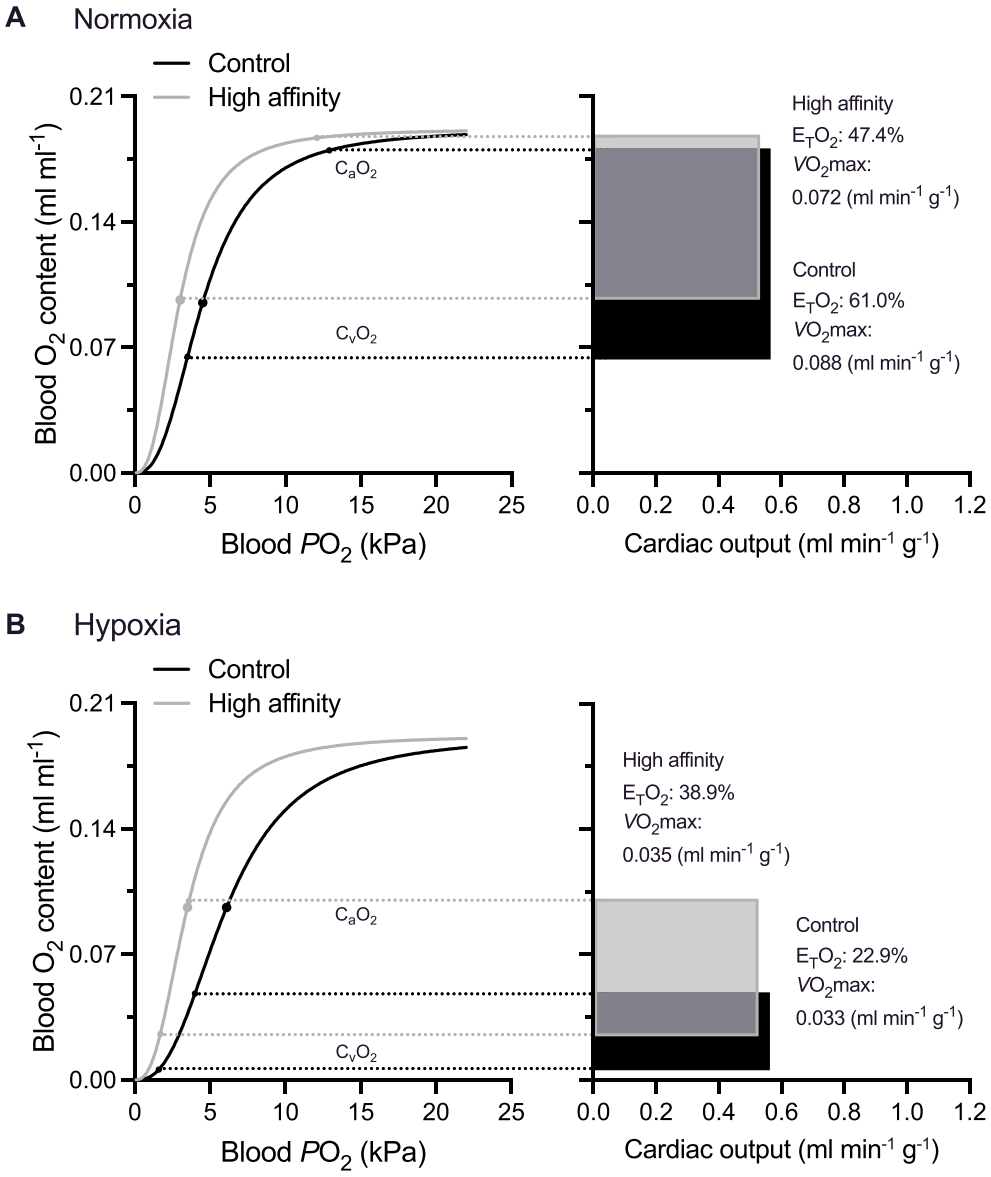

Fig. 3. Johansen plots showing how traits that govern circulatory O_2 delivery and tissue O_2 extraction contribute to $\dot{V}O_{2max}$ in Sprague-Dawley rats. Data are shown for rats tested in normoxia (A) and hypoxia (B). Left-hand panels show O_2-equilbrium curves based on data for the control group (solid black line) and the treatment group with pharmacologically increased Hb-O_2 affinity (solid grey line). In both A and B, the area of the box in the right-hand panel denotes $\dot{V}O_{2max}$ elicited by aerobic exercise (value inside the box in ml min^{-1} g^{-1}). Graphs were plotted using data from experimental groups 2 and 3 in Henderson et al. (2000).

hypoxia, it is possible that the increased Hb-O_2 affinity would confer a greater performance advantage if the associated increase in maximal convective O_2 delivery (the product of C_aO_2 and cardiac output) more than offsets the decrease in tissue O_2 extraction. This would be consistent with results of experiments that documented a significantly higher survival rate of cyanate-treated rats relative to controls when exposed to a barometric pressure of 29.7 kPa (=223 torr, equivalent to an altitude of >9000 m) (Eaton et al., 1974). Nonetheless, results of these experiments show that a left-shifted O_2-equilibrium curve negatively affects $\dot{V}O_{2max}$ over a broad range of PO_2 values when [Hb] is experimentally held constant. The limiting factor is O_2 diffusion from capillary blood to muscle cells, as indicated by a strong, positive correlation between $\dot{V}O_{2max}$ and P_vO_2 in the full set of experimental animals (McCanse et al., 1999; Henderson et al., 2000).

3.2. Thermogenic performance in highland and lowland deer mice with evolved differences in Hb-O_2 affinity

North American deer mice (*Peromyscus maniculatus*) are distributed from sea level to altitudes >4300 m and common-garden experiments have revealed that highland natives have evolved enhanced performance capacities in hypoxia in comparison with lowland relatives (Storz et al., 2019). Highland natives have higher capacities for both aerobic thermogenesis and exercise in hypoxia due to a combination of evolved and plastic changes in numerous respiratory, cardiovascular, and metabolic traits (Cheviron et al., 2012, 2014; Lui et al., 2015; Scott et al., 2015, 2018; Ivy and Scott, 2017, 2018; Lau et al., 2017; Mahalingam et al., 2017, 2020; Tate et al., 2017, 2020; Dawson et al., 2018; Nikel et al., 2018; Storz et al., 2019; Ivy et al., 2020).

In hypoxia, the higher thermogenic $\dot{V}O_{2max}$ of highlanders relative to lowlanders is largely attributable to increased cardiac output (Fig. 4), a trait that exhibits an accentuated plastic response to hypoxia exposure in highland mice (Tate et al., 2017, 2020). Lowland mice exhibit a higher increase in [Hb] during hypoxia acclimation in comparison with highland mice, which accounts for the higher upper asymptote of the O_2-equilbrium curve shown in Fig. 4. However, highland mice maintain a higher S_aO_2 in hypoxia, which minimizes the decline in C_aO_2 caused by the reduction in P_aO_2. Common garden experiments revealed that the highland vs. lowland difference in S_aO_2 is genetically based, and appears to stem largely from differences in Hb-O_2 affinity (Tate et al., 2017, 2020; Ivy et al., 2020).

Highland deer mice have evolved an increased Hb-O_2 affinity in comparison to lowland conspecifics and closely related congeners such as *P. leucopus* (Storz et al., 2009, 2010a; Natarajan et al., 2015a; Jensen et al., 2016; Ivy et al., 2020). Protein-engineering experiments have identified and functionally characterized causative mutations in the α- and β-chains of the $\alpha_2\beta_2$ Hb tetramer, and have provided detailed insights into the biophysical mechanisms responsible for the affinity-enhancing effects (Natarajan et al., 2013). The key question is whether the genetically based changes in Hb-O_2 affinity translate into changes in whole-animal physiological performance in hypoxia? If so, are the changes in blood P_{50} sufficient to improve hypoxic $\dot{V}O_{2max}$ on their own, or do they only confer a physiological benefit in conjunction with changes in other traits? Previous work involving reciprocal-transplant experiments involving wild-derived strains of deer mice revealed that high-altitude natives with high Hb-O_2 affinities have higher $\dot{V}O_{2max}$ in hypoxia in comparison with low-altitude conspecifics (Chappell and Snyder, 1984; Chappell et al., 1988). This documented association between Hb-O_2 affinity and hypoxic $\dot{V}O_{2max}$ provides physiological context for interpreting altitudinal patterns of allele frequency variation in the underlying globin genes (Snyder et al., 1988; Storz et al., 2009, 2010a, 2012; Natarajan et al., 2015a).

To identify intermediate links in the chain of causation between Hb function and whole-animal aerobic performance, Wearing et al. (2021) produced interpopulation hybrids of highland and lowland mice using an F_2 intercross breeding design. This design effectively randomizes associations between allelic α- and β-globin variants against an admixed genetic background. They then examined phenotypic effects of the Hb variants on cardiorespiratory and hematological traits (measured *in vivo*) and thermogenic $\dot{V}O_{2max}$ in hypoxia. These experiments confirmed that the elevated Hb-O_2 affinity contributes to an enhanced S_aO_2 in hypoxia (Tate et al., 2017, 2020), but – in contrast to previous studies – they revealed no statistically significant association with thermogenic $\dot{V}O_{2max}$ in hypoxia (Wearing et al., 2021). One possible explanation for the apparent discrepancy is that the experiments of Chappell and colleagues tested the effects of affinity-enhancing Hb variants on a 'highland' background, whereas Wearing et al. (2021) tested the effects of similar variants on admixed highland/lowland backgrounds. It is therefore possible that the physiological effects of affinity-enhancing Hb variants are conditional on changes in other phenotypes that are characteristic of highland mice and that were not manifest in the admixed population examined by Wearing et al. (2021). To investigate this possibility, Wearing et al. (2021) used the experimental data to parameterize an in silico model of the O_2-transport pathway and measured main effects and interaction effects of Hb-O_2 affinity and tissue O_2 diffusion capacity (D_TO_2) on $\dot{V}O_{2max}$. Specifically, they used a sensitivity analysis to determine the effects of increasing D_TO_2 on $\dot{V}O_{2max}$ at Hb-O_2 affinities characteristic of mice with representative highland and lowland α/β Hb

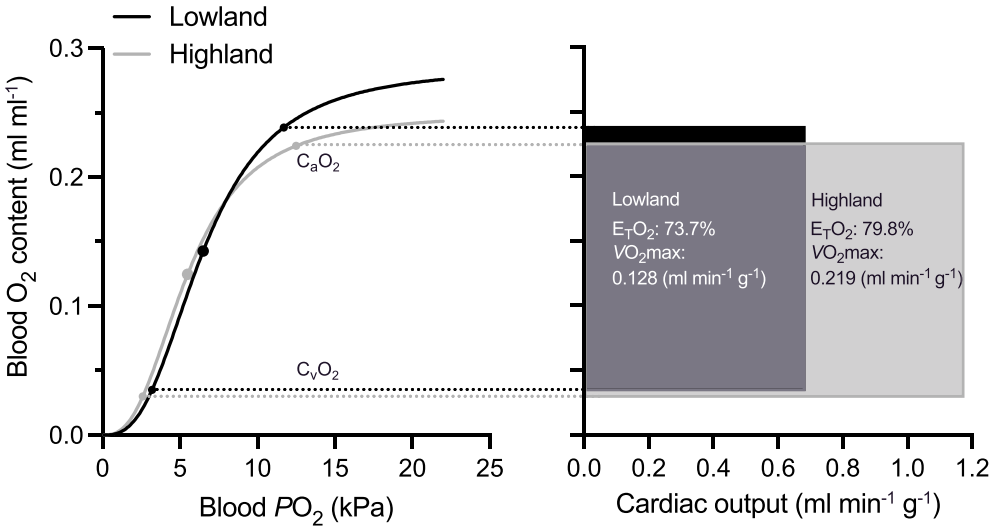

Fig. 4. Johansen plot showing how traits that govern circulatory O_2 delivery and tissue O_2 extraction contribute to hypoxic $\dot{V}O_{2max}$ in highland deer mice (*Permomyscus maniculatus*) and lowland white-footed mice (*P. leucopus*). The left-hand panel shows O_2-equilbrium curves based on data for highland deer mice (grey) and lowland white-footed mice (black). In the right-hand panel, the area of the box denotes thermogenic $\dot{V}O_{2max}$ elicited by cold exposure (value inside the box in ml min^{-1} g^{-1}). Replicated from Wearing and Scott (2021) and based on experimental data from Tate et al. (2020) and Ivy et al. (2020).

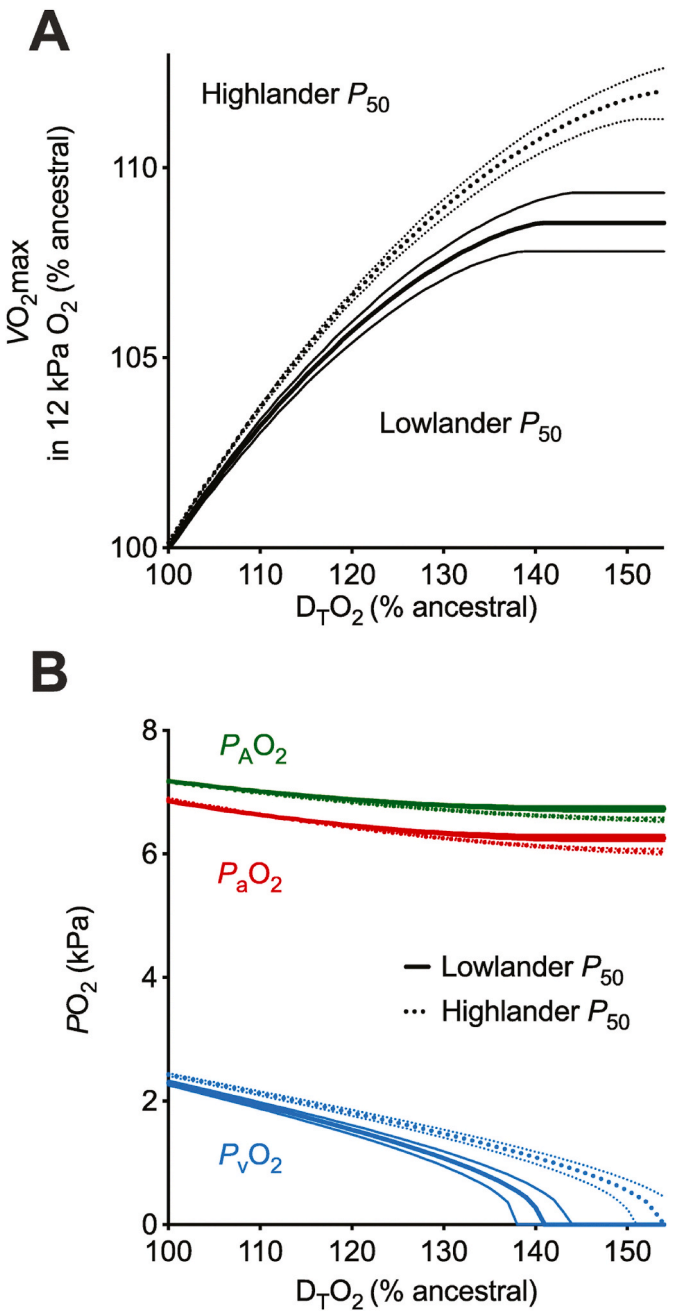

Fig. 5. Effects of increasing tissue O_2 diffusing capacity (D_TO_2) on hypoxic $\dot{V}O_{2max}$ and blood PO_2's inferred from mathematical modeling of the O_2-transport pathway in deer mice. (A) Relative changes in hypoxic $\dot{V}O_{2max}$ and (B) changes in alveolar (P_AO_2), arterial (P_aO_2), and venous (P_vO_2) in response to relative increases in D_TO_2. Effects were modeled using blood P_{50}'s of deer mice with representative high- and low-altitude Hb genotypes (mean values and standard errors of the mean are denoted with bold and fine lines, respectively). Reproduced from Wearing et al. (2021).

genotypes. Results revealed that increasing D_TO_2 by 50% increases $\dot{V}O_{2max}$, but the effect was greater in mice with the high-affinity Hb genotype (10.7%) than in mice with the low-affinity genotype (7.7%) (Fig. 5A). The effect of Hb-O_2 affinity was accentuated when D_TO_2 was increased above 37%, when P_vO_2 (and thus venous O_2 saturation) fell to zero at the lower Hb-O_2 affinity (=higher P_{50}) (Fig. 5B). Results of this study suggest that increases in Hb-O_2 affinity only contribute to the enhancement of hypoxic $\dot{V}O_{2max}$ if accompanied by a corresponding increase in tissue O_2 diffusion capacity. Importantly, highland deer mice have evolved changes in muscle microvasculature and mitochondrial oxidative capacity that should increase tissue O_2 diffusion capacity (Lui et al., 2015; Scott et al., 2015, 2018; Mahalingam et al., 2017, 2020; Tate et al., 2017, 2020; Nikel et al., 2018). Thus, available data suggest that genetically based increases in both Hb-O_2 affinity and tissue O_2 diffusion capacity contributed to the enhanced aerobic capacity of high-altitude deer mice in hypoxia. The experimental and modeling results also demonstrate how the adaptive value of changes in one trait may be conditional on changes in other traits that govern different steps in the O_2-transport pathway (Wearing et al., 2021; Wearing and Scott 2021).

3.3. *Exercise performance in humans with high-affinity Hb mutants*

To test whether an elevated Hb-O_2 affinity improves aerobic performance in hypoxia, Dominelli et al. (2020) conducted exercise trials involving human subjects with high-affinity Hb mutants and a control group with normal Hb. The high-affinity Hb (HAH) subjects possessed Hb Malmö (β97His→Gln) or Hb San Diego (β109Val→Met), with standard P_{50}'s of 2.0–2.3 kPa (15–17 torr) compared to 3.5 kPa (26 torr) for wildtype human Hb. The exercise trials involved acute exposure to an atmosphere of 15% O_2 (equivalent to an altitude of ~3000 m) and test subjects did not undergo any prior acclimation.

In both the HAH and control groups, hypoxia exposure resulted in an expected reduction in exercise performance compared to that achieved in normoxia. However, the HAH group exhibited a significantly attenuated decline in $\dot{V}O_{2max}$ relative to controls (4 ± 5% vs 12 ± 5% decline). The attenuated decline in the HAH group was not explained by changes in ventilation, heart rate, or P_aO_2 relative to the control group. Instead, the HAH group had a significantly higher S_aO_2 in hypoxia, which translated into a greater C_aO_2. Subjects with the lowest decrements in S_aO_2 had the lowest decrement in hypoxic $\dot{V}O_{2max}$, as indicated by the significant negative correlation between the change in S_aO_2 and the change in $\dot{V}O_{2max}$ (Fig. 6). The HAH group also exhibited a higher [Hb] relative to controls. At sea level, polycythemia is commonly observed in subjects with high-affinity Hb mutants, as it represents a compensatory erythropoietic response to impaired tissue oxygenation (Shepherd et al., 2019). Although the elevated [Hb] of HAH subjects also contributed to the augmentation of C_aO_2 in hypoxia, there was no significant correlation between [Hb] and $\dot{V}O_{2max}$ in the HAH group or in the control group. It thus appears that the superior performance of the HAH group in hypoxia is primarily attributable to the maintenance of a higher S_aO_2, which in turn prevents C_aO_2 from falling more than it would otherwise. The resultant enhancement of blood O_2 conductance presumably more than offsets the associated impairment in tissue O_2 unloading.

In contrast with the study of human HAH subjects with Hb Malmö and Hb San Diego (Dominelli et al., 2020), a similar human study that tested the effects of pharmacologically increasing Hb-O_2 affinity detected no net effect on $\dot{V}O_{2max}$ (Stewart et al., 2021). One possible explanation for the discrepancy between the two studies is that the HAH subjects in the study by Dominelli et al. (2020) were born with high-affinity Hbs and they had a lifetime to develop compensatory physiological adjustments (possibly involving pulmonary gas exchange or tissue O_2 diffusion capacity). In other words, HAH subjects were internally acclimated to the possession of high-affinity Hb. By contrast, acute increases in Hb-O_2 affinity that are achieved via pharmacological manipulations, as in the study by Stewart et al. (2021), do not permit long-term adjustments in interrelated respiratory or cardiovascular traits. Consequently, it may be that the two studies tested biochemically analogous modifications of Hb function in different physiological contexts.

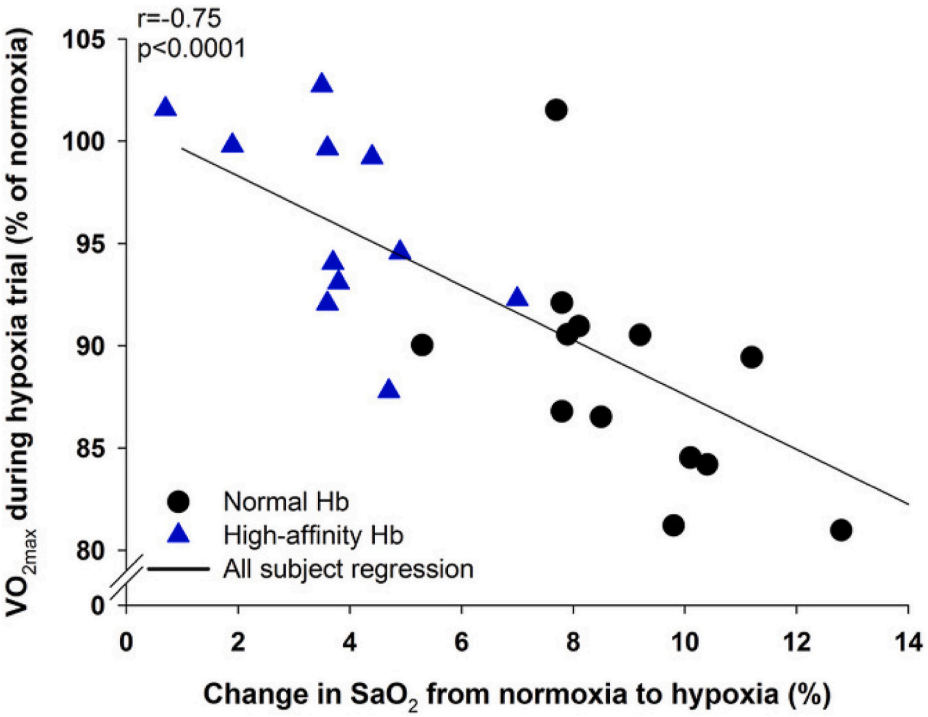

Fig. 6. Relationship between the change in $\dot{V}O_{2max}$ between exercise trials in normoxia vs. hypoxia and the corresponding change in oxyHb saturation. Data are for human subjects with normal or high-affinity Hbs. Reproduced with permission from Dominelli et al. (2020).

4. Conclusions

The case studies involving rats, deer mice, and humans provide insights into the combination of hematological changes that can contribute to the enhancement of convective O_2 transport in hypoxia, thereby minimizing the expected decrement in aerobic performance in situations where $\dot{V}O_{2max}$ is limited by O_2 supply. Such insights suggest hypotheses about the phenotypic changes that we might expect to observe in highland species that are presumably well-adapted to chronic hypoxia. In the case of [Hb] – a highly labile, hypoxia-responsive trait – observed patterns of altitudinal variation among populations or species may not tell us much about how the phenotypic optimum varies according to ambient PO_2, as observed [Hb] values may be broadly reflective of overall levels of tissue oxygenation and stimulus-response characteristics of the erythropoietin system. By contrast, observed patterns of altitudinal variation in Hb-O_2 affinity among populations or species are potentially informative about how optimal P_{50} varies as a function of ambient PO_2, provided that the measured values reflect genetically based variation.

Under conditions of severe hypoxia, modeling results suggest that an increased Hb-O_2 affinity may improve aerobic performance if it helps safeguard S_aO_2, but only if it is accompanied by an increased tissue O_2 diffusion capacity so that the augmentation of C_aO_2 translates into a corresponding enhancement of tissue O_2 extraction. This insight informs expectations about patterns of phenotypic correlations in nature and predicts that high-altitude species that exhibit an increased Hb-O_2 affinity will also exhibit changes in muscle microvasculature and/or oxidative capacity that enhance tissue O_2 diffusion capacity.

Declaration of competing interest

None.

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Abbreviations

[Hb]	hemoglobin concentration
C_aO_2	arterial O_2 content
C_vO_2	venous O_2 content
P_aO_2	arterial partial pressure of O_2
P_AO_2	alveolar partial pressure of O_2
P_vO_2	venous partial pressure of O_2
P_{50}	partial pressure of O_2 at which hemoglobin is half saturated
βbO_2	blood capacitance coefficient
Q	cardiac output
$\dot{V}O_{2max}$	maximum rate of O_2 consumption
S_aO_2	arterial O_2 saturation
D_TO_2	Tissue O_2 diffusion capacity

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