



# Influence of High Hemoglobin-Oxygen Affinity on Humans During Hypoxia

Kevin L. Webb<sup>1</sup>, Paolo B. Dominelli<sup>2</sup>, Sarah E. Baker<sup>1</sup>, Stephen A. Klassen<sup>1,3</sup>, Michael J. Joyner<sup>1</sup>, Jonathon W. Senefeld<sup>1†</sup> and Chad C. Wiggins<sup>1\*†</sup>

<sup>1</sup> Department of Anesthesiology and Perioperative Medicine, Mayo Clinic, Rochester, MN, United States, <sup>2</sup> Department of Kinesiology, University of Waterloo, Waterloo, ON, Canada, <sup>3</sup> Department of Kinesiology, Brock University, St. Catharines, ON, Canada

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### \*Correspondence:

Chad C. Wiggins  
Wiggins.Chad@mayo.edu

<sup>†</sup>These authors have contributed  
equally to this work

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Humans elicit a robust series of physiological responses to maintain adequate oxygen delivery during hypoxia, including a transient reduction in hemoglobin-oxygen (Hb-O<sub>2</sub>) affinity. However, high Hb-O<sub>2</sub> affinity has been identified as a beneficial adaptation in several species that have been exposed to high altitude for generations. The observed differences in Hb-O<sub>2</sub> affinity between humans and species adapted to high altitude pose a central question: is higher or lower Hb-O<sub>2</sub> affinity in humans more advantageous when O<sub>2</sub> availability is limited? Humans with genetic mutations in hemoglobin structure resulting in high Hb-O<sub>2</sub> affinity have shown attenuated cardiorespiratory adjustments during hypoxia both at rest and during exercise, providing unique insight into this central question. Therefore, the purpose of this review is to examine the influence of high Hb-O<sub>2</sub> affinity during hypoxia through comparison of cardiovascular and respiratory adjustments elicited by humans with high Hb-O<sub>2</sub> affinity compared to those with normal Hb-O<sub>2</sub> affinity.

**Keywords:** altitude acclimatization, high-altitude, oxygen transport, exercise,  $\dot{V}O_{2\max}$  (maximal oxygen uptake), high affinity hemoglobin (Hb)

## INTRODUCTION

Currently, there is ongoing debate about the advantages of higher or lower hemoglobin-oxygen (Hb-O<sub>2</sub>) affinity in humans, particularly during hypoxia (Dempsey, 2020). A decrease in Hb-O<sub>2</sub> affinity is often observed among humans during acclimatization to altitudes ranging from 2500 to 4500 m, presumably to facilitate O<sub>2</sub> off-loading and protect against tissue hypoxia (Hall et al., 1936; Aste-Salazar and Hurtado, 1944; Lenfant and Sullivan, 1971). In contrast, several animal species adapted to high-altitude environments display a higher Hb-O<sub>2</sub> affinity compared to that of low-land counterparts (Bartels et al., 1963; Monge and Leon-Velarde, 1991; Weber et al., 1993; Scott and Milsom, 2007; Storz et al., 2010; Storz, 2016; Natarajan et al., 2018). These divergent observations lead to the central question of this review, is higher or lower Hb-O<sub>2</sub> affinity more advantageous for humans during hypoxia?

Humans rely on a continuous supply of O<sub>2</sub> for metabolism. Oxygen binds to hemoglobin in the lungs and travels through the large arteries, arterioles, and finally the small capillaries supplying peripheral tissue (Scholander, 1960). Although *in vitro* Hb-O<sub>2</sub> affinity is characterized by a single curve or metric (e.g., P<sub>50</sub>, as described below), the *in vivo* Hb-O<sub>2</sub> affinity cannot be described as

simply. Within the vasculature, alterations of modulatory factors such as temperature, pH, and the concentration of carbon dioxide (CO<sub>2</sub>) lead to transient changes in Hb-O<sub>2</sub> affinity during circulatory transit, which directly impact O<sub>2</sub> loading at the lung and O<sub>2</sub> off-loading in peripheral tissue (Jensen, 2004; Winslow, 2007). Changes in Hb-O<sub>2</sub> affinity can be transient or chronic due to a variety of conditions such as genetic mutations, disease, altitude acclimatization, or age (Woodson et al., 1970; Humpeler and Amor, 1973; Versmold et al., 1973; Winslow, 2007). For example, evidence suggests that some groups of humans native to high altitude have a greater Hb-O<sub>2</sub> affinity than sea-level residents (Simonson et al., 2014; Li et al., 2018). Although the mechanisms underlying the adaptive increase of Hb-O<sub>2</sub> affinity among high-altitude natives are not well understood, a number of genetic mutations in hemoglobin structure that contribute to a systemic increase in Hb-O<sub>2</sub> affinity in humans have been identified (Mangin, 2017), predominantly among low-altitude residents. Humans with mutations resulting in high Hb-O<sub>2</sub> affinity may provide unique insight to the ongoing debate regarding the advantages and disadvantages of high Hb-O<sub>2</sub> affinity during hypoxia. Past investigations of the cardiorespiratory adjustments to hypoxic exposure at rest and during exercise suggest that high Hb-O<sub>2</sub> affinity may provide better maintenance of O<sub>2</sub> delivery and utilization in humans. Therefore, the purpose of this review is to highlight the potential advantages and disadvantages of high Hb-O<sub>2</sub> affinity in humans during hypoxia through examination of cardiovascular and respiratory adjustments at rest and during exercise.

To address the central question of this review, we examine available studies reporting cardiovascular or respiratory adjustments to hypoxia at rest or during exercise in humans with genetic mutations resulting in high Hb-O<sub>2</sub> affinity. To avoid confounding factors that may alter cardiovascular and respiratory responses, we excluded studies in which these individuals have recently undergone venesection. Studies fitting these criteria can be found in **Table 1**, including participant characteristics and experimental design. To clearly denote the “severity” of hypoxia within the discussion, we define low altitude as <2500 m, high altitude as >2500 m, and extreme altitude as >7000 m.

## FOUNDATIONAL CONCEPTS

Hemoglobin-oxygen affinity is largely determined by the structure of hemoglobin and modulated by a variety of factors within the vasculature [temperature, pH, CO<sub>2</sub>, 2,3-diphosphoglycerate (2,3-DPG), organic phosphates, chloride ions (Cl<sup>-</sup>), etc.] (Mairbäurl et al., 1993). The relationship between the partial pressure of O<sub>2</sub> (P<sub>O<sub>2</sub></sub>) and O<sub>2</sub> saturation can be described by the O<sub>2</sub> dissociation curve (**Figure 1**). One common metric to quantify Hb-O<sub>2</sub> affinity is P<sub>50</sub>, defined as the P<sub>O<sub>2</sub></sub> at which 50% of hemoglobin is saturated with O<sub>2</sub>. A lower P<sub>50</sub> corresponds to a higher Hb-O<sub>2</sub> binding affinity or a “left-shifted” O<sub>2</sub> dissociation curve. On the other hand, a higher P<sub>50</sub> corresponds to a lower Hb-O<sub>2</sub> binding affinity and a “right-shifted” O<sub>2</sub> dissociation curve. In addition to P<sub>50</sub>,

the Hill coefficient is often used to describe the curvature of the O<sub>2</sub> dissociation curve (Endrenyi et al., 1975; Piiper, 1992; Riggs, 1998). However, describing the O<sub>2</sub> dissociation curve with the P<sub>50</sub> and the Hill coefficient presents some limitations. Experimentally, the P<sub>50</sub> and Hill coefficient are commonly determined using *in vitro* standardized environmental conditions [pH ~7.4, partial pressure of CO<sub>2</sub> (P<sub>CO<sub>2</sub></sub>) ~40 mmHg, and temperature ~37°C], which does not account for transient changes in the *in vivo* modulation of Hb-O<sub>2</sub> affinity during circulatory transit (Braumann et al., 1982). Therefore, there is not “one” O<sub>2</sub> dissociation curve because the binding affinity and cooperativity of hemoglobin vary throughout the vasculature. Nevertheless, standardized measurements of P<sub>50</sub> and the Hill coefficient allow general inter-individual comparisons of Hb-O<sub>2</sub> affinity, but do not account for *in vivo* modulation of Hb-O<sub>2</sub> affinity.

Changes in Hb-O<sub>2</sub> affinity throughout the vasculature optimize both O<sub>2</sub> loading in the lungs and O<sub>2</sub> off-loading to peripheral tissue. For example, byproducts of metabolism (increased temperature, increased CO<sub>2</sub>, and lower pH) contribute to a localized decrease in Hb-O<sub>2</sub> affinity in exercising muscle, thereby promoting O<sub>2</sub> off-loading and utilization (Böning et al., 1975). Furthermore, a lower temperature and increased pH within the lung result in a localized increase in Hb-O<sub>2</sub> affinity and improved O<sub>2</sub> loading (Mairbäurl, 2013). Alternatively, long-term regulation of modulatory factors or alterations in the structure of hemoglobin can lead to systemic wide changes in Hb-O<sub>2</sub> affinity. For instance, hypoxia increases 2,3-DPG concentration (due to increased glycolytic activity) in red blood cells contributing to a systemic decrease of Hb-O<sub>2</sub> affinity (Lenfant et al., 1968). Standard teaching supports that a decrease in Hb-O<sub>2</sub> affinity facilitates O<sub>2</sub> off-loading during hypoxia (Hall et al., 1936; Aste-Salazar and Hurtado, 1944). Yet, the systemic decrease in Hb-O<sub>2</sub> affinity would compromise O<sub>2</sub> loading in the lung, particularly when O<sub>2</sub> availability is limited during hypoxia. At higher altitudes, a decrease in Hb-O<sub>2</sub> affinity would be even more disadvantageous and further compromised O<sub>2</sub> loading would likely impede peripheral O<sub>2</sub> delivery. Conversely, an increase in Hb-O<sub>2</sub> affinity during hypoxia promotes O<sub>2</sub> loading within the lungs and mitigates reductions in arterial O<sub>2</sub> saturation (Eaton et al., 1974; Yalcin and Cabrales, 2012). In addition, the advantages conferred by increased Hb-O<sub>2</sub> affinity are augmented at higher altitudes, outweighing potential limitations in O<sub>2</sub> off-loading (Eaton et al., 1974). Therefore, homeostatic maintenance of O<sub>2</sub> delivery and utilization during hypoxia is contingent on the balance between O<sub>2</sub> loading in the lungs and O<sub>2</sub> off-loading in the periphery, both of which are largely determined by the Hb-O<sub>2</sub> affinity. Additional discussion of hemoglobin structure and the regulation of Hb-O<sub>2</sub> affinity is presented below (see section “Hemoglobin-Oxygen Affinity”).

## Hemoglobin-Oxygen Affinity

Hemoglobin is a tetramer consisting of two α-subunits and two β-subunits (Coates, 1975). Each subunit contains a heme group that is capable of reversibly binding O<sub>2</sub> (Perutz, 1963). When hemoglobin is fully saturated four O<sub>2</sub> molecules are bound independently to each of the four

**TABLE 1** | Studies examining cardiorespiratory adjustments during normoxia or hypoxia in humans with high Hb-O<sub>2</sub> affinity.

Study	Age (years)	Sex (n)	Hb type	P <sub>50</sub> (mmHg)	[Hb] (g/dL)	Hct (%)	Study design
Hebbel et al., 1977	12	1M	Hb Andrew-Minneapolis	17	16	NR	Hypoxic ventilatory response (F <sub>I</sub> O <sub>2</sub> = 0.13, ~3800 m)
	18	1F	Hb Andrew-Minneapolis	17	17	NR	
Hebbel et al., 1978	12	1M	Hb Andrew-Minneapolis	17	17	48	High-altitude acclimatization (~3100 m) and graded cycling to exhaustion
	18	1F	Hb Andrew-Minneapolis	17	17	50	
Rossoff et al., 1980	25	2M	Hb Rainier	12	NR	NR	Hypoxic ventilatory response (F <sub>I</sub> O <sub>2</sub> = 0.14, ~3300 m)
Wranne et al., 1983	30	1M	NR	14	19	55	Normoxic submaximal cycling
	31	1M	NR	14	18	54	
Länsimies et al., 1985	38 (14)	5M	Hb Linköping	16 (0.4)	19 (1)	NR	Normoxic graded cycling to exhaustion
	32 (8)	5F	Hb Linköping	17 (0.5)	16 (4)	NR	
Dominelli et al., 2019	45 (8)	3M	Hb Malmö	15 (0.2)	21 (1)	63 (3)	Hypoxic ventilatory response (F <sub>I</sub> O <sub>2</sub> = 0.14, ~3300 m)
	43 (15)	6F	Hb Malmö (n = 5), Hb San Diego (n = 1)	16 (1.1)	19 (1)	55 (3)	
Dominelli et al., 2020	45 (8)	3M	Hb Malmö	15 (0.2)	21 (1)	63 (3)	Normoxic and normobaric hypoxic (F <sub>I</sub> O <sub>2</sub> = 0.15, ~2600 m) graded cycling to exhaustion
	31 (9)	8F	Hb Malmö (n = 7), Hb San Diego (n = 1)	16 (0.9)	18 (1)	54 (2)	

The fraction of inspired O<sub>2</sub> and associated elevation are provided under study design.

Abbreviations: M, male; F, female; Hb, hemoglobin; P<sub>50</sub>, the P<sub>O<sub>2</sub></sub> at which 50% of hemoglobin is saturated with O<sub>2</sub>; Hb, hemoglobin; Hct, hematocrit; F<sub>I</sub>O<sub>2</sub>, fraction of inspired O<sub>2</sub>; NR, not reported. Numbers within parentheses throughout the table indicate standard deviations.

subunits of the hemoglobin molecule. Hemoglobin undergoes a conformational shift with each O<sub>2</sub> molecule that binds, existing in a T (tense) state when deoxygenated and a R (relaxed) state when oxygenated, commonly described by a two-state model (Monod et al., 1965). As each individual subunit becomes oxygenated, a conformational shift further increases binding affinity for O<sub>2</sub> (Mihailescu and Russu, 2001). This cooperativity in O<sub>2</sub> binding to hemoglobin gives rise to the sigmoidal shape of the O<sub>2</sub> dissociation curve (Figure 1).

Hemoglobin is subject to allosteric regulation by multiple ligands. Most notably, higher concentrations of H<sup>+</sup> and CO<sub>2</sub> reduce Hb-O<sub>2</sub> affinity (Riggs, 1960; Ho and Russu, 1987). This pH dependent change of Hb-O<sub>2</sub> affinity is termed the Bohr effect (Bohr et al., 1904). Hb-O<sub>2</sub> affinity is also reduced at higher temperatures (Weber and Campbell, 2011). For example, an increase of temperature from 37 to 40°C raises P<sub>50</sub> from normal values of ~27 to 30 mmHg (Hlastala et al., 1977). Additionally, the magnitude of the Bohr effect is greater at higher temperatures, further promoting O<sub>2</sub> off-loading from hemoglobin (Hlastala et al., 1977). The cooperative effect of a more acidic environment along with higher temperatures, as occurs during rigorous exercise, significantly reduces Hb-O<sub>2</sub> affinity such that P<sub>50</sub> may increase up to ~40 mmHg within the vasculature (Thomson et al., 1974). In the case of severe respiratory alkalosis, a fivefold

increase in minute ventilation may reduce arterial P<sub>CO<sub>2</sub></sub> from normal values of ~40 mmHg to as low as 7 mmHg and blood pH may exceed 7.7 (Houston et al., 1987; West, 2006). At extreme altitudes, *in vivo* P<sub>50</sub> may be reduced to less than 20 mmHg due to changes in blood P<sub>CO<sub>2</sub></sub> and pH (West, 1984; Winslow et al., 1984).

The erythrocytic concentrations of 2,3-DPG and Cl<sup>-</sup> are associated with more long-term modulation of Hb-O<sub>2</sub> affinity. In effect, 2,3-DPG and Cl<sup>-</sup> bind to deoxygenated hemoglobin and stabilize the T state, reducing Hb-O<sub>2</sub> affinity (Benesch et al., 1967; Brewer, 1974). 2,3-DPG reduces Hb-O<sub>2</sub> affinity and increases the cooperativity of hemoglobin, which “right-shifts” the O<sub>2</sub> dissociation curve and steepens the slope (Tyuma et al., 1971). In addition, an influx of Cl<sup>-</sup> into the red blood cell, coupled to the outward transport of bicarbonate, reduces Hb-O<sub>2</sub> affinity (Wieth et al., 1982; Perutz et al., 1994; Prange et al., 2001). These ligands elicit independent effects on Hb-O<sub>2</sub> affinity, and complex *in vivo* interactions between ligands give rise to the physiological P<sub>50</sub> of hemoglobin. For example, 2,3-DPG and Cl<sup>-</sup> compete for binding to hemoglobin and the effect 2,3-DPG on Hb-O<sub>2</sub> affinity disappears at high concentrations of Cl<sup>-</sup> (Imai, 1982). In addition, the Bohr effect is more pronounced at greater concentrations of 2,3-DPG (Bauer, 1969). The interested reader may consult other sources for more detailed discussions on modulation of Hb-O<sub>2</sub> affinity (Antonini and Brunori, 1970; Mairbaurl and Weber, 2012).

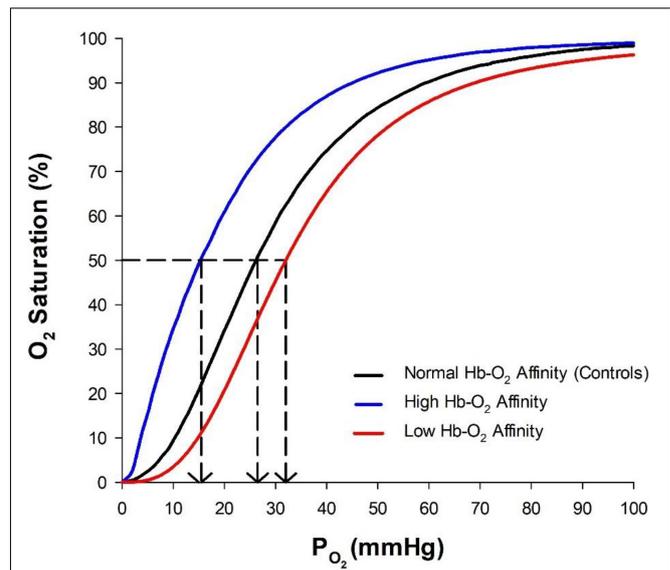
The severity and duration of hypoxia is an important factor when considering *in vivo* modulation of Hb-O<sub>2</sub> affinity in humans. During sojourns to altitudes of ~4500 m or less, humans demonstrate a reduced Hb-O<sub>2</sub> affinity due to elevated production of 2,3-DPG (Lenfant et al., 1968). At these elevations, hyperventilation reduces blood P<sub>CO<sub>2</sub></sub> and potentially results in respiratory alkalosis (Dempsey and Forster, 1982). However, renal compensation leads to the excretion of excess bicarbonate and conservation of H<sup>+</sup>, normalizing blood pH to sea-level values after a few days at high altitude (Goldfarb-Rumyantzev and Alper, 2014; Bird et al., 2021). At higher elevations (4500–5400 m), hyperventilation becomes so pronounced that renal compensation is insufficient and blood pH increases (West, 2006). The rise in blood pH increases Hb-O<sub>2</sub> affinity, counteracting the effects of an elevated 2,3-DPG production such that P<sub>50</sub> approximates values observed at sea-level (Mairbaurl and Weber, 2012). As humans travel above ~5400 m, Hb-O<sub>2</sub> affinity increases as the respiratory alkalosis becomes more severe (West, 1984).

Contemporary studies suggest a potential role of hemoglobin found in cells other than erythrocytes such as alveolar epithelial cells, lung cells, and mesangial cells (Du et al., 2012; Saha et al., 2014). Within these non-erythrocytic cells, the production of hemoglobin appears to be upregulated in response to hypoxia (Cheung et al., 1997; Tezel et al., 2009; Grek et al., 2011), potentially serving as a “reservoir” for O<sub>2</sub> (Saha et al., 2014). Therefore, a key area for future investigation is the relationship between non-erythroid hemoglobin production and hypoxia tolerance. However, there is currently minimal evidence to suggest that non-erythroid hemoglobin provides a functional impact on cardiovascular adjustments during hypoxia.

## Pharmacological Induction of High Hemoglobin-Oxygen Affinity

Several pharmacological methods which transfuse 2,3-DPG depleted red blood cells into both animals and humans have allowed investigation into the role of high Hb-O<sub>2</sub> affinity in O<sub>2</sub> transport (Riggs et al., 1973; Woodson et al., 1973; Wranne et al., 1974; Bakker et al., 1976; Malmberg et al., 1979; Woodson and Auerbach, 1982; Birchard and Tenney, 1991). However, methods used to achieve 2,3-DPG depletion often alter acid-base balance and total blood volume, potentially confounding the observed cardiorespiratory adjustments (Birchard and Tenney, 1991). More recent developments of pharmaceuticals that induce high Hb-O<sub>2</sub> affinity allow examination of altered Hb-O<sub>2</sub> affinity with fewer complications (Dufu et al., 2017; Kalfa et al., 2019; Stewart et al., 2020, 2021). For example, voxelotor binds allosterically to some, but not all hemoglobin and increases Hb-O<sub>2</sub> affinity. Hemoglobin modified with voxelotor exhibits a reduced Bohr effect compared to unmodified hemoglobin (Pochron et al., 2019), which may limit O<sub>2</sub> off-loading during instances where blood pH decreases such as rigorous exercise.

In general, allosteric modifiers allow for the manipulation of Hb-O<sub>2</sub> affinity with less perturbations in acid-base balance associated with 2,3-DPG depletion techniques. However, in



**FIGURE 1** | Oxygen dissociation curve showing normal hemoglobin-O<sub>2</sub> (Hb-O<sub>2</sub>) affinity (P<sub>50</sub> ~26 mmHg), high Hb-O<sub>2</sub> affinity (P<sub>50</sub> ~16 mmHg), and low Hb-O<sub>2</sub> affinity (P<sub>50</sub> ~32 mmHg). The P<sub>50</sub>, denoted by the dashed lines, is defined as the P<sub>O<sub>2</sub></sub> at which 50% of hemoglobin is saturated with O<sub>2</sub>.

healthy humans voxelotor induces only a modest decrease in P<sub>50</sub> of ~2 mmHg (Stewart et al., 2020, 2021) compared to the greater range from 3 to 10 mmHg obtained *via* 2,3-DPG depletion (Gillette et al., 1974; Wranne et al., 1974). The ability to pharmacologically alter Hb-O<sub>2</sub> affinity in humans both acutely and chronically may provide additional insights on the context-dependent circumstances at which high Hb-O<sub>2</sub> affinity is advantageous (i.e., magnitude and duration of hypoxia).

## Humans With High Hemoglobin-Oxygen Affinity Hemoglobinopathies

Currently, over 200 distinct mutations resulting in high Hb-O<sub>2</sub> affinity have been identified (Charache et al., 1966; Mangin, 2017). By definition, high Hb-O<sub>2</sub> affinity is characterized by a P<sub>50</sub> less than 24 mmHg (Figure 1; Rumi et al., 2009; Mangin, 2017). However, a majority of high Hb-O<sub>2</sub> affinity hemoglobinopathies examined are associated with P<sub>50</sub> values ranging from 12 to 17 mmHg (Table 1). Both the amino acid substitution and location at which the substitution occurs within the hemoglobin molecule may affect Hb-O<sub>2</sub> affinity, cooperativity, and response to modulatory ligands. Within the hemoglobin mutations represented in this review (Table 1), all exhibit reduced cooperativity and only Hb Andrew-Minneapolis demonstrates a reduced Bohr effect (Adamson et al., 1969; Boyer et al., 1972; Nute et al., 1974; Zak et al., 1974; Wranne et al., 1983; Berlin et al., 2009). Lower cooperativity gives rise to the unique shape of the standard O<sub>2</sub> dissociation curve in humans with high Hb-O<sub>2</sub> affinity (Figure 1). However, the complex interactions between modulatory factors and subsequent effects on *in vivo* Hb-O<sub>2</sub> affinity have not been clearly elucidated in mutated hemoglobin molecules.

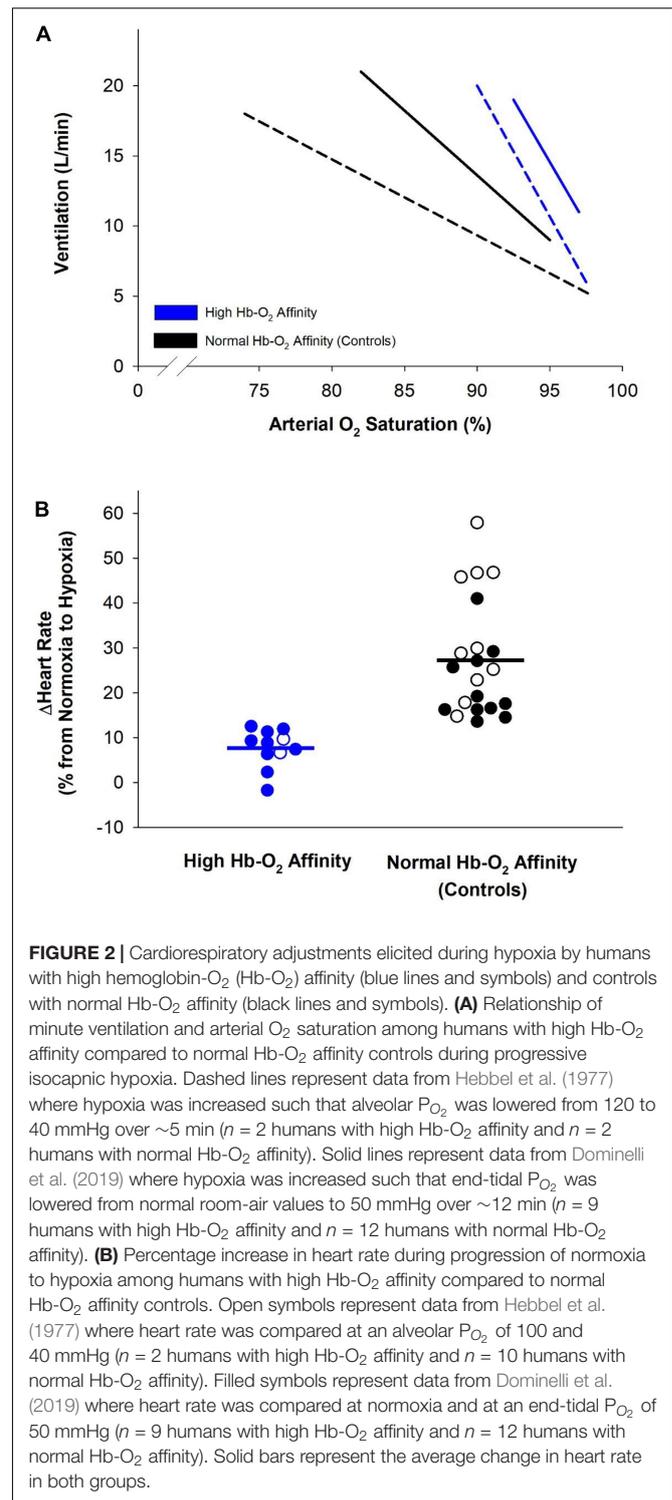
Due to a lower P<sub>50</sub>, O<sub>2</sub> off-loading is likely compromised in those with high Hb-O<sub>2</sub> affinity. Evidence for compromised O<sub>2</sub> off-loading may be seen through compensatory increases in hematocrit resulting in a higher O<sub>2</sub> carrying capacity per unit of blood (Charache et al., 1966; Mangin, 2017; Shepherd et al., 2019). It is thought that the kidneys sense a reduction of O<sub>2</sub> off-loading and promote red blood cell production in response, functioning as a “critmeter” (Donnelly, 2001). In addition to an elevated hematocrit humans with high Hb-O<sub>2</sub> affinity likely develop skeletal muscle adaptations to compromised O<sub>2</sub> off-loading such as a greater percentage of non-oxidative (type II) muscle fibers than their counterparts with normal Hb-O<sub>2</sub> affinity (Wranne et al., 1983). Additionally, a greater accumulation of metabolic byproducts (e.g., lactate and H<sup>+</sup>) during high-intensity exercise have been reported in humans with high Hb-O<sub>2</sub> affinity compared to those with normal Hb-O<sub>2</sub> affinity (Länsimies et al., 1985; Dominelli et al., 2020). Those with high Hb-O<sub>2</sub> affinity demonstrate a similar lactate accumulation at the end of exhaustive exercise during both normoxia and hypoxia, whereas controls demonstrate a reduced lactate accumulation during hypoxia compared to normoxia (Dominelli et al., 2020). A possible explanation for these observations may be that humans with high Hb-O<sub>2</sub> affinity obtain similar power outputs in normoxia and hypoxia and therefore demonstrate a similar metabolite accumulation between the two conditions; whereas those with normal Hb-O<sub>2</sub> affinity have a reduced power output and lower lactate concentrations during hypoxia compared to normoxia.

The observed differences in skeletal muscle fiber composition and utilization of metabolic pathways supporting exercise between humans with high Hb-O<sub>2</sub> affinity and humans with normal Hb-O<sub>2</sub> affinity may be due to differences in O<sub>2</sub> off-loading kinetics and tissue P<sub>O<sub>2</sub></sub> (Wranne et al., 1983). In general, many physiological compensatory responses coinciding with high Hb-O<sub>2</sub> affinity remain uncharacterized. Key areas for future investigation include adaptations to high Hb-O<sub>2</sub> affinity possibly affecting capillary density, blood flow distribution, and skeletal muscle aerobic capacity (Dempsey, 2020).

## HIGH HEMOGLOBIN-OXYGEN AFFINITY AND CARDIORESPIRATORY ADJUSTMENTS DURING HYPOXIA AT REST

### Acute Hypoxia

Brief periods of hypoxia require both cardiovascular and respiratory adjustments to maintain adequate O<sub>2</sub> delivery (Rowell and Blackmon, 1987; Bärttsch and Saltin, 2008; Naeije, 2010). One crucial immediate adjustment in response to hypoxia is increased ventilation which raises alveolar ventilation, increases arterial P<sub>O<sub>2</sub></sub> and protects against arterial O<sub>2</sub> desaturation (Otis et al., 1956; Dempsey and Forster, 1982). At a given alveolar P<sub>O<sub>2</sub></sub>, humans with high Hb-O<sub>2</sub> affinity have similar minute ventilation compared to humans with normal Hb-O<sub>2</sub> affinity (Hebbel et al., 1977; Rossoff et al., 1980; Dominelli



**FIGURE 2 |** Cardiorespiratory adjustments elicited during hypoxia by humans with high hemoglobin-O<sub>2</sub> (Hb-O<sub>2</sub>) affinity (blue lines and symbols) and controls with normal Hb-O<sub>2</sub> affinity (black lines and symbols). **(A)** Relationship of minute ventilation and arterial O<sub>2</sub> saturation among humans with high Hb-O<sub>2</sub> affinity compared to normal Hb-O<sub>2</sub> affinity controls during progressive isocapnic hypoxia. Dashed lines represent data from Hebbel et al. (1977) where hypoxia was increased such that alveolar P<sub>O<sub>2</sub></sub> was lowered from 120 to 40 mmHg over ~5 min (*n* = 2 humans with high Hb-O<sub>2</sub> affinity and *n* = 2 humans with normal Hb-O<sub>2</sub> affinity). Solid lines represent data from Dominelli et al. (2019) where hypoxia was increased such that end-tidal P<sub>O<sub>2</sub></sub> was lowered from normal room-air values to 50 mmHg over ~12 min (*n* = 9 humans with high Hb-O<sub>2</sub> affinity and *n* = 12 humans with normal Hb-O<sub>2</sub> affinity). **(B)** Percentage increase in heart rate during progression of normoxia to hypoxia among humans with high Hb-O<sub>2</sub> affinity compared to normal Hb-O<sub>2</sub> affinity controls. Open symbols represent data from Hebbel et al. (1977) where heart rate was compared at an alveolar P<sub>O<sub>2</sub></sub> of 100 and 40 mmHg (*n* = 2 humans with high Hb-O<sub>2</sub> affinity and *n* = 10 humans with normal Hb-O<sub>2</sub> affinity). Filled symbols represent data from Dominelli et al. (2019) where heart rate was compared at normoxia and at an end-tidal P<sub>O<sub>2</sub></sub> of 50 mmHg (*n* = 9 humans with high Hb-O<sub>2</sub> affinity and *n* = 12 humans with normal Hb-O<sub>2</sub> affinity). Solid bars represent the average change in heart rate in both groups.

et al., 2019). Yet, due to the left-shifted nature of their oxygen dissociation curve, those with high Hb-O<sub>2</sub> affinity have a higher arterial O<sub>2</sub> saturation at a given alveolar P<sub>O<sub>2</sub></sub> (Figure 2A; Hebbel et al., 1977; Rossoff et al., 1980; Dominelli et al., 2019).

In addition to increased ventilation, hypoxia is associated with increased cardiac output, primarily through an elevated

heart rate (Brown and Grocott, 2013; Siebenmann and Lundby, 2015). As arterial O<sub>2</sub> saturation decreases during hypoxia, cardiac output increases and peripheral arterioles dilate to match O<sub>2</sub> delivery and demand (Ekblom et al., 1975; Phillips et al., 1988). These observations suggest that the change in heart rate during acute hypoxia is closely linked to systemic O<sub>2</sub> delivery (Casey and Joyner, 2011; Joyner and Casey, 2014; Siebenmann and Lundby, 2015). During acute hypoxia, humans with high Hb-O<sub>2</sub> affinity display a lesser increase in heart rate, and presumably cardiac output, likely due to better maintained arterial O<sub>2</sub> content (**Figure 2B**; Hebbel et al., 1977; Dominelli et al., 2019). Since arterial O<sub>2</sub> saturation remains fairly constant in humans with high Hb-O<sub>2</sub> affinity during modest reductions of P<sub>O<sub>2</sub></sub>, as occurs at moderately high altitude, arterial O<sub>2</sub> content is better maintained and heart rate increases to a lesser extent compared to those with normal Hb-O<sub>2</sub> affinity.

Peripheral chemosensors located at both the carotid and aortic bodies respond to acute changes in arterial P<sub>O<sub>2</sub></sub> and P<sub>CO<sub>2</sub></sub>, such as during normobaric and hypobaric hypoxia (Lahiri and Forster, 2003). Stimulation of peripheral chemosensors during hypoxic exposure causes an increase in minute ventilation and sympathetic activity in an attempt to maintain O<sub>2</sub> homeostasis (Powell et al., 1998; Bernardi et al., 2001; Fletcher, 2001). Examination of humans with high Hb-O<sub>2</sub> affinity provides support for low P<sub>O<sub>2</sub></sub> being a strong stimulus in the hypoxic ventilatory response, rather than arterial O<sub>2</sub> saturation or content (Hebbel et al., 1977; Rossoff et al., 1980; Dominelli et al., 2019). Some evidence suggests that aortic chemosensors sense changes in arterial O<sub>2</sub> content and heart rate is adjusted accordingly (Lugliani et al., 1971; Wasserman, 1978; Lahiri et al., 1980, 1981). Therefore, the lower heart rate during hypoxia among humans with high Hb-O<sub>2</sub> affinity compared to controls may be caused by decreased sensory stimulus of the aortic chemosensors (Dominelli et al., 2019). However, the mechanistic stimulation of the peripheral chemosensors requires that O<sub>2</sub> be dissociated from hemoglobin to be sensed (Lopez-Barneo et al., 2001). Therefore, the relationship between O<sub>2</sub> content and P<sub>O<sub>2</sub></sub> sensed at the carotid chemosensors remains unclear and contention exists regarding mechanisms of O<sub>2</sub> sensing and regulation of systemic blood flow (Ward, 2008). Detailed discussions into the mechanism of O<sub>2</sub> sensing are provided elsewhere (Lopez-Barneo et al., 2001; Kumar and Prabhakar, 2012).

The observed relationship between ventilation and arterial O<sub>2</sub> saturation may present a disadvantage to humans with high Hb-O<sub>2</sub> affinity during acute hypoxic exposure. Since the stimulus for ventilation is closely linked to arterial P<sub>O<sub>2</sub></sub> and not arterial O<sub>2</sub> saturation during brief periods of hypoxia (Biscoe, 1971; Guz, 1975; Weil and Zwillich, 1976), humans with high Hb-O<sub>2</sub> affinity have an excessive ventilatory response despite only a modest drop in arterial O<sub>2</sub> saturation and delivery (**Figure 2A**). Excessive ventilation increases O<sub>2</sub> consumption by respiratory muscles (Cherniack, 1959; Robertson et al., 1977). Although accounting for a small percentage of total O<sub>2</sub> consumption during rest, respiratory muscle O<sub>2</sub> demand increases during hyperventilation or exercise (Aaron et al., 1992; Coast et al., 1993; Dominelli et al., 2015). Thus, during exercise, there are increased and competitive demands for O<sub>2</sub> in metabolically active

tissue including both exercising muscle and respiratory muscle (Harms et al., 2000; Sheel et al., 2001; Romer and Polkey, 2008; Dominelli et al., 2017). This competition for blood flow between respiratory and exercising muscle limits exercise tolerance at higher and extreme altitudes and is often referred to as “respiratory steal” (Pugh et al., 1964; Schoene, 2001; Helfer et al., 2016). The physiological consequences of “respiratory steal” are likely exacerbated at more extreme altitudes as hyperventilation, and thus metabolic demand of respiratory muscle, becomes more pronounced. Therefore, the excessive hyperventilation during acute hypoxic exposure may be disadvantageous for humans with high Hb-O<sub>2</sub> affinity due to increased O<sub>2</sub> consumption by respiratory muscles with minimal improvement in arterial O<sub>2</sub> saturation.

## Chronic Hypoxia

In addition to acute hypoxic exposure, the benefits of high Hb-O<sub>2</sub> affinity have been observed through examination of cardiorespiratory adjustments during 10-days of residing at high altitude (Leadville, Colorado, ~3100 m elevation) (Hebbel et al., 1978). Two humans with high Hb-O<sub>2</sub> affinity and two of their siblings with normal Hb-O<sub>2</sub> affinity were examined during the acclimatization period. Changes in arterial 2,3-DPG concentration and pH were similar during the stay at high altitude in both sets of siblings. However, peak and average heart rate during acclimatization were lower in the siblings with high Hb-O<sub>2</sub> affinity. During hypoxia, impaired O<sub>2</sub> delivery to the kidneys prompts erythropoietin production (Donnelly, 2001; Nangaku and Eckardt, 2007; Haase, 2013). Erythropoietin stimulates red blood cell production and leads to a subsequent increase in O<sub>2</sub> carrying capacity to compensate for impaired O<sub>2</sub> delivery (Erslev, 1991; Jelkmann, 2011). Humans with high Hb-O<sub>2</sub> affinity showed smaller increases in erythropoietin production when residing at high altitude (Hebbel et al., 1978). A lesser erythropoietin production during high-altitude acclimatization suggests that O<sub>2</sub> delivery is better preserved among humans with high Hb-O<sub>2</sub> affinity. Similarly, Hall et al. (1936) showed that mammals native to high altitude display a reduced erythropoietic response during travel from low altitude to high altitude. Combined, these findings suggest that lessened cardiovascular adjustments are needed to maintain adequate O<sub>2</sub> delivery during high-altitude acclimatization in humans with high Hb-O<sub>2</sub> affinity compared to those with normal Hb-O<sub>2</sub> affinity.

Marked physiological compensations are required to maintain homeostasis during sojourn to extreme altitudes (West, 2006). Hb-O<sub>2</sub> affinity increases at altitudes greater than ~5400 m due to severe respiratory alkalosis with insufficient renal compensations (see section “Hemoglobin-Oxygen Affinity”). During ascent to the summit of Mt. Everest, ~8100 m, climbers had a reduction in P<sub>50</sub> from ~26 mmHg to less than ~20 mmHg (West, 1984). A more recent study examining blood oxygenation of four climbers reported arterial saturations ranging from 34 to 70% at the summit of Everest (Grocott et al., 2009). Without an increase of Hb-O<sub>2</sub> affinity due to respiratory alkalosis it is likely that humans would not be able to reach the summit without supplemental O<sub>2</sub>.

As extreme altitude challenges the ability to transport O<sub>2</sub> from atmospheric air to tissue, the modulation of Hb-O<sub>2</sub> affinity is crucial to maintain adequate O<sub>2</sub> consumption. Enhanced O<sub>2</sub> loading in the lungs due to high Hb-O<sub>2</sub> affinity is even more advantageous at extreme altitude than at high altitude, where ambient P<sub>O<sub>2</sub></sub> can fall to as low as 40 mmHg, outweighing potential limitations in O<sub>2</sub> off-loading (Eaton et al., 1974). The ventilatory response during hypoxia is similar between humans with genetic mutations leading to high Hb-O<sub>2</sub> affinity and those with normal Hb-O<sub>2</sub> affinity (Hebbel et al., 1977; Rossoff et al., 1980; Dominelli et al., 2019). Under the circumstances of extreme altitude, humans with high Hb-O<sub>2</sub> affinity may develop respiratory alkalosis to a similar degree as observed in humans with normal Hb-O<sub>2</sub> affinity (West, 1984; Grocott et al., 2009). In addition, some genetic hemoglobin mutations demonstrate a preserved Bohr effect, such that Hb-O<sub>2</sub> affinity would decrease during respiratory alkalosis by a similar magnitude compared to non-mutated hemoglobin (Adamson et al., 1969; Boyer et al., 1972; Nute et al., 1974; Wranne et al., 1983; Berlin et al., 2009). A physiological consequence of respiratory alkalosis would be further left-shifted O<sub>2</sub> dissociation curve adding additional protection against arterial desaturation. Therefore, humans with genetic modifications resulting in high Hb-O<sub>2</sub> affinity and a preserved Bohr effect may ascend to extreme altitudes with fewer physiological complications (i.e., Acute mountain sickness, high-altitude cerebral edema, and impaired cognitive function) compared to sojourners with normal Hb-O<sub>2</sub> affinity. However, to our knowledge no humans with genetic high Hb-O<sub>2</sub> affinity have been examined at altitudes greater than ~3100 m and the proposed physiologic responses to higher and extreme altitudes are theoretical.

Groups of indigenous humans who have resided at high altitude for many generations display genotypic and phenotypic adaptations to the hypoxic environment (Beall, 2007, 2014; Moore, 2017; Tymko et al., 2019; Storz, 2021). Recent evidence has suggested an adaptive increase of Hb-O<sub>2</sub> affinity among high altitude natives of the Qinghai-Tibetan Plateau (>3500 m) compared to sea-level residents (Simonson et al., 2014; Li et al., 2018). However, others have reported that some high-altitude populations [Nepalese (>3800 m), Peruvian (>4500 m), and Qinghai-Tibetan (>3500 m) natives] do not show this adaptive increase in Hb-O<sub>2</sub> affinity (Samaja et al., 1979; Winslow et al., 1981; Tashi et al., 2014). Additional studies may improve understanding of changes in Hb-O<sub>2</sub> affinity observed among high-altitude natives and molecular mechanisms underlying such adaptation.

The Qinghai-Tibetan natives had a P<sub>50</sub> ~2 mmHg lower than the sea-level residents (24.5 vs. 26.2 mmHg, respectively) (Simonson et al., 2014). However, the high-altitude natives did not display improvements in pulmonary gas exchange or peak exercise capacity during hypoxia compared to the sea-level residents, suggesting no clear benefit of high Hb-O<sub>2</sub> affinity in the population examined. These findings, contradictory to those observed in humans with genetic mutations resulting in high Hb-O<sub>2</sub> affinity, could be explained by differences in the magnitude of P<sub>50</sub>. The high-altitude natives studied had a P<sub>50</sub> of ~25 mmHg, in contrast to values ranging from 12 to 17 mmHg

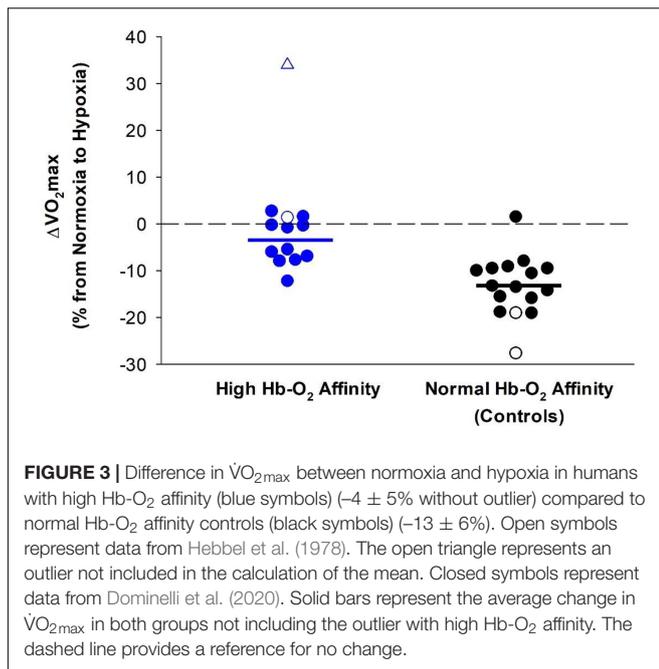
observed in humans with genetic mutations resulting in high Hb-O<sub>2</sub> affinity (Table 1). Therefore, the P<sub>50</sub> observed in the high-altitude native population is probably not low enough to warrant significant alterations in pulmonary gas exchange, O<sub>2</sub> extraction, and exercise capacity during hypoxia. In addition, adaptations of high-altitude populations, which affect multiple steps within the O<sub>2</sub> transport cascade (Beall, 2007), may confound our ability to clearly dissociate the role of increased Hb-O<sub>2</sub> affinity in humans native to high altitude.

## HIGH HEMOGLOBIN-OXYGEN AFFINITY AND CARDIORESPIRATORY ADJUSTMENTS DURING EXERCISE

### Maximal Oxygen Consumption During Normoxia

Studies examining the effects of pharmacologically induced high Hb-O<sub>2</sub> affinity on O<sub>2</sub> consumption during normoxia have provided discordant results in both humans and animals (Riggs et al., 1973; Woodson et al., 1973; Wranne et al., 1974; Valeri et al., 1975; Yhap et al., 1975; Bakker et al., 1976; Malmberg et al., 1979; Ross and Hlastala, 1981; Woodson and Auerbach, 1982; Stewart et al., 2020, 2021). Recently, Stewart et al. (2021) showed that pharmaceutical induction of high Hb-O<sub>2</sub> affinity (only ~2 mmHg decrease in P<sub>50</sub>) using voxelotor reduced normoxic maximal O<sub>2</sub> consumption ( $\dot{V}O_{2max}$ ) in humans. The decrement in normoxic  $\dot{V}O_{2max}$  observed by Stewart et al. (2021) could be due to both an increase in Hb-O<sub>2</sub> affinity and a reduced Bohr effect: the transient reduction of Hb-O<sub>2</sub> affinity with decreasing pH (Pochron et al., 2019). A reduced Bohr effect in exercising muscle would further compromise O<sub>2</sub> off-loading, particularly during periods of high metabolic demand (Mairbörl, 2013). Conversely, some mathematical models suggest that normoxic  $\dot{V}O_{2max}$  is relatively insensitive to modest increases in Hb-O<sub>2</sub> affinity despite limitations in O<sub>2</sub> off-loading (Wagner, 1997; Shepherd et al., 2019).

In corroboration with results found through mathematical modeling, humans with high Hb-O<sub>2</sub> affinity show no difference in normoxic  $\dot{V}O_{2max}$  values compared to similar age, sex-matched controls with normal Hb-O<sub>2</sub> affinity (Länsimies et al., 1985; Dominelli et al., 2020). However, there is evidence for altered metabolic processes among humans with high Hb-O<sub>2</sub> affinity compared to controls during exercise testing. During cycling exercise in normoxia, humans with high Hb-O<sub>2</sub> affinity may have greater reliance on anaerobic metabolism during heavy to maximal exercise, as evidenced by lower blood pH and pronounced lactate production compared to controls (Wranne et al., 1983; Länsimies et al., 1985; Dominelli et al., 2020). In addition, humans with high Hb-O<sub>2</sub> affinity seem to display a worsened exercise efficiency during cycling, i.e., higher O<sub>2</sub> consumption for a given power output (Dominelli et al., 2020). Theoretically, compromised O<sub>2</sub> off-loading due to high Hb-O<sub>2</sub> affinity may give rise to the greater reliance on anaerobic metabolism, which contributes to the worsened exercise efficiency observed (Dominelli et al., 2020). In brief,



current evidence indicates that humans with high Hb-O<sub>2</sub> affinity have similar normoxic  $\dot{V}O_{2\max}$  values despite altered metabolic processes during high-intensity exercise.

Little is known about the relationship between high Hb-O<sub>2</sub> affinity and compensatory mechanisms that facilitate adequate O<sub>2</sub> extraction. Wranne et al. (1983) demonstrated that the arterial-venous O<sub>2</sub> extraction was abnormally low during exercise in humans with high Hb-O<sub>2</sub> affinity, suggesting that O<sub>2</sub> off-loading may be compromised within muscle during whole-body exercise. However, humans with high Hb-O<sub>2</sub> affinity had a ~25% greater O<sub>2</sub> carrying capacity than those with normal Hb-O<sub>2</sub> affinity, likely compensating for the diminished arterial-venous O<sub>2</sub> extraction both at rest and during exercise. The potential benefits of high Hb-O<sub>2</sub> affinity are likely contingent on the capacity to extract O<sub>2</sub> from blood (Wearing et al., 2021). The capacity of O<sub>2</sub> off-loading and diffusion to the mitochondria are crucial to maximize O<sub>2</sub> utilization in cases of high Hb-O<sub>2</sub> affinity, especially during peak whole-body exercise. Therefore, future research should focus on the relationship between high Hb-O<sub>2</sub> affinity and compensatory mechanisms which facilitate adequate O<sub>2</sub> extraction within peripheral tissue such as alterations in the microvascular architecture, flow of the red blood cells through the microvasculature, and the diffusion gradients driving O<sub>2</sub> to the mitochondria.

## Maximal Oxygen Consumption During Hypoxia

Maximal O<sub>2</sub> consumption in humans decreases with increasing severity of hypoxia (Faulkner et al., 1968; Grover, 1970; Lawler et al., 1988; Ferretti et al., 1997; Wehrin and Hallén, 2006; Wagner, 2010; West, 2010). However, humans with high Hb-O<sub>2</sub> affinity are better able to maintain  $\dot{V}O_{2\max}$  during hypoxia compared to those with normal Hb-O<sub>2</sub> affinity (Figure 3).

As previously described, Hebbel and colleagues examined four siblings, two with high Hb-O<sub>2</sub> affinity and two with normal Hb-O<sub>2</sub> affinity, during 10 days of high-altitude acclimatization (Leadville, Colorado, ~3100 m elevation). At high altitude  $\dot{V}O_{2\max}$  decreased by ~28 and 19% compared to sea-level values in the two siblings with normal Hb-O<sub>2</sub> affinity (Hebbel et al., 1978). On the other hand, the two siblings with high Hb-O<sub>2</sub> affinity did *not* demonstrate a reduction in  $\dot{V}O_{2\max}$  at high altitude compared to low altitude (Hebbel et al., 1978).

Similarly, experiments using acute normobaric hypoxia showed that humans with high Hb-O<sub>2</sub> affinity had better maintained  $\dot{V}O_{2\max}$  during hypoxia compared to humans with normal Hb-O<sub>2</sub> affinity (Dominelli et al., 2020). In addition, peak power output during cycling exercise was better preserved in those with high Hb-O<sub>2</sub> affinity (Dominelli et al., 2020). At both high altitude and normobaric hypoxia, there was no difference in maximal heart rate during exercise in humans with high Hb-O<sub>2</sub> affinity compared to those with normal Hb-O<sub>2</sub> affinity (Hebbel et al., 1978; Dominelli et al., 2020). Previous studies indicate that an increase in blood viscosity associated with an elevated hematocrit, common in humans with chronic high Hb-O<sub>2</sub> affinity, may limit blood flow and maximal cardiac output in humans (Richardson and Guyton, 1959; Schumacker et al., 1985; Çınar et al., 1999). On the contrary, some studies suggest that systemic blood flow at rest and during exercise within animals is not reduced at a hematocrit of ~50–60% (Gaehtgens et al., 1979; Schumacker et al., 1985; Lindenfeld et al., 2005). However, hematocrits greater than 60% likely result in a substantially elevated blood viscosity such that systemic blood flow is restricted (Weisse et al., 1964; Gaehtgens et al., 1979; Schumacker et al., 1985). Therefore, it is unclear whether cardiac output and systemic blood flow is limited among humans with high Hb-O<sub>2</sub> affinity where hematocrit often ranges from ~55 to 65%.

The reduction of  $\dot{V}O_{2\max}$  during hypoxia is directly related to the degree of arterial desaturation (Hughes et al., 1968; Calbet et al., 2003a). As such, a higher arterial O<sub>2</sub> saturation in humans with high Hb-O<sub>2</sub> affinity for a given level of hypoxia likely contributes to the preservation of hypoxic  $\dot{V}O_{2\max}$  (Figure 3). In humans with normal Hb-O<sub>2</sub> affinity at high altitude, hypoxic  $\dot{V}O_{2\max}$  is less than values measured at sea-level and hypoxic  $\dot{V}O_{2\max}$  either remains the same or progressively increases during acclimatization (Saltin et al., 1968; Calbet et al., 2003b). Despite acclimatization, hypoxic  $\dot{V}O_{2\max}$  does not reach values previously measured at sea-level (Calbet et al., 2003b). In contrast, humans with high Hb-O<sub>2</sub> affinity have a better maintained hypoxic  $\dot{V}O_{2\max}$  upon transition to high altitude, but it is unknown how humans with high Hb-O<sub>2</sub> affinity may acclimatize to high altitude and subsequent effects on hypoxic  $\dot{V}O_{2\max}$ .

## CONCLUSION

High Hb-O<sub>2</sub> affinity has been identified as a potentially advantageous adaptation to high altitude in several animal species. From a cardiorespiratory perspective, we suggest that high Hb-O<sub>2</sub> affinity is advantageous for humans when exposed

to hypoxic environments both at rest and during exercise. During hypoxia, humans with high Hb-O<sub>2</sub> affinity exhibit lessened increases in heart rate, reduced erythropoietin production, and higher arterial O<sub>2</sub> saturation at rest compared to those with normal Hb-O<sub>2</sub> affinity. In addition,  $\dot{V}O_{2\max}$  and work capacity are better maintained during hypoxia compared to normoxia in humans with high Hb-O<sub>2</sub> affinity. The advantages associated with high Hb-O<sub>2</sub> affinity are likely potentiated as the degree of hypoxia becomes more severe. In addition, high Hb-O<sub>2</sub> affinity confers physiological disadvantages at less severe magnitudes of hypoxia such as reduced O<sub>2</sub> off-loading and unwarranted hyperventilation when arterial O<sub>2</sub> saturation is fairly well-preserved. However, current understanding on the effects of high Hb-O<sub>2</sub> affinity during hypoxia is largely limited to normobaric hypoxia. Future research warrants the investigation into the influence of high Hb-O<sub>2</sub> affinity during both short- and long-term periods of high-altitude acclimatization. In addition, long-term adaptations to pharmacologically induced high Hb-O<sub>2</sub> affinity in humans remains largely unexamined. Regardless, the influence of high Hb-O<sub>2</sub> affinity on cardiorespiratory adjustments to environmental hypoxia is of key interest in

human adaptation to environmental hypoxia, particularly during bouts of exercise.

## AUTHOR CONTRIBUTIONS

MJ and CW conceived the concept for this review. KW, JS, and CW drafted the manuscript. PD, SB, JS, SK, and MJ provided critical revision of the manuscript for important intellectual content. All authors approved the final version of the manuscript.

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