The adaptive benefit of increases in hemoglobin-O_2 affinity is contingent on tissue O_2 diffusing capacity in high-altitude deer mice

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Author Contributions

G.R.S., J.F.S., and Z.A.C. designed the study. O.H.W., C.M.I., J.P.V., N.G.-P., S.C.C.-S., and C.N. ran the experiments. O.H.W. analyzed the data. O.H.W. and G.R.S. wrote the manuscript, and all authors edited the manuscript.

Abstract

Complex organismal traits are often the result of multiple interacting genes and sub-organismal phenotypes, but how these interactions shape the evolutionary trajectories of adaptive traits is poorly understood. We examined how functional interactions between cardiorespiratory traits contribute to adaptive increases in the capacity for aerobic thermogenesis (maximal O_2 consumption, $\dot{V}O_2$max, during acute cold exposure) in high-altitude deer mice (*Peromyscus maniculatus*). We crossed highland and lowland deer mice to produce F_2 inter-population hybrids, which expressed genetically based variation in hemoglobin (Hb) O_2 affinity on a mixed genetic background. We then combined physiological experiments and mathematical modeling of the O_2 transport pathway to examine links between cardiorespiratory traits and $\dot{V}O_2$max. Physiological experiments revealed that increases in Hb-O_2 affinity of red blood cells improved blood oxygenation in hypoxia, but were not associated with enhancements in $\dot{V}O_2$max. Sensitivity analyses performed using mathematical modeling showed that the influence of Hb-O_2 affinity on $\dot{V}O_2$max in hypoxia was contingent on the capacity for O_2 diffusion in active tissues. These results suggest that increases in Hb-O_2 affinity would only have adaptive value in hypoxic conditions if concurrent with or preceded by increases in tissue O_2 diffusing capacity. In high-altitude deer mice, the adaptive benefit of increasing Hb-O_2 affinity is contingent on the capacity to extract O_2 from the blood, which helps resolve controversies about the general role of hemoglobin function in hypoxia tolerance.

Significance Statement

Complex organismal traits are often the result of multiple interacting genes and phenotypes, but the role of these interactions in shaping adaptive traits is poorly understood. We combined physiological experiments and modeling to examine how functional interactions between cardiorespiratory traits underlie high-altitude adaptation in deer mice. We show that adaptive increases in thermogenic capacity result from a functional interaction between blood hemoglobin and active tissues, in which the adaptive benefit of increasing hemoglobin O_2 affinity is contingent on the capacity for O_2 diffusion from the blood. This helps reconcile controversy about the general role of hemoglobin in hypoxia tolerance, and provides insight into physiological mechanisms of high-altitude adaptation.

Main Text

Introduction

A long-standing goal of evolutionary biology is to understand how the functional integration of traits influences patterns of phenotypic change and adaptation (1). Complex physiological phenotypes often represent an emergent property of functional interactions among different tissues and organ systems, which in turn may be developmentally interrelated and genetically correlated. The functional, developmental, and genetic interdependence of traits may facilitate environmental adaptation if semi-autonomous components of a complex phenotype respond synergistically to selection. Alternatively, functional integration and genetic correlations among components of a trait can limit and channel pathways of phenotypic evolution (2, 3). Evolutionary questions about phenotypic integration and adaptation can be addressed most profitably by examining well-defined traits with well-characterized functions and well-documented associations with fitness under natural conditions.

The capacity for aerobic thermogenesis in small mammals at high altitude is a complex performance trait that is well suited to experimental studies of how patterns of phenotypic integration affect the process of adaptation. At high altitude, cold temperatures challenge the ability of endotherms to maintain body temperature and activity, which is especially difficult in smaller animals that have high surface area to volume ratios. Unsurprisingly, aerobic thermogenesis (quantified as maximal oxygen consumption, $\dot{V}O_2max$, during acute cold exposure) in hypoxia is under strong directional selection in some small mammals at high altitude (4), which have evolved higher thermogenic $\dot{V}O_2max$ (5-9). Thermogenic $\dot{V}O_2max$ is supported by the integrated function of the O_2 transport pathway, the conceptual steps (ventilation, pulmonary diffusion, circulation, tissue diffusion, and mitochondrial O_2 utilization) involved in transporting O_2 from inspired air to thermogenic tissues where O_2 is used by mitochondria to support oxidative phosphorylation (10, 11). Therefore, studies of thermogenic $\dot{V}O_2max$ in high-altitude natives are ideal for understanding the mechanisms underlying the adaptive evolution of complex traits.

Evolved increases in the O_2 affinity of hemoglobin (Hb) are pervasive in high-altitude taxa, and have become classic examples of biochemical adaptation (12). However, the nature of the direct adaptive benefit conferred by increases in Hb-O_2 affinity in highland species is controversial. Many highland taxa have evolved increases in Hb-O_2 affinity independently, and in many cases, the molecular mechanisms underlying these changes in protein function are documented in detail (12-18). These increases in Hb-O_2 affinity are often presumed to safeguard arterial O_2 saturation in hypoxia and thus help improve tissue O_2 delivery and aerobic capacity (5, 6, 19-26), although this has rarely been tested. Nonetheless, the relationship between Hb-O_2 affinity and $\dot{V}O_2max$ in hypoxia remains contentious (12, 25, 27-29). Theoretical modeling of the O_2 transport pathway in humans suggests that increases in Hb-O_2 affinity do not increase aerobic capacity in hypoxia on their own (30), because the advantage of increasing Hb-O_2 affinity may be offset by a trade-off in O_2 offloading at tissues (11, 31, 32). A recent study in humans with rare genetic Hb variants found that increases in Hb-O_2 affinity attenuated the hypoxia-induced decline in aerobic capacity, but subjects with high Hb-O_2 affinity also had compensatory polycythemia (33). Considering the strong functional integration of Hb within the O_2 transport pathway, the advantages of increasing Hb-O_2 affinity in high-altitude taxa may be contingent on the evolution of other cardiorespiratory traits, but this has not been experimentally investigated.

We sought to determine the effects of evolved increases in Hb-O_2 affinity in high-altitude deer mice (*Peromyscus maniculatus*) on thermogenic $\dot{V}O_2max$ in hypoxia, and to examine whether the adaptive benefit of changes in Hb-O_2 affinity is contingent on other cardiorespiratory changes. Deer mice have the broadest altitudinal range of any North American mammal (34), and high-altitude populations have evolved elevated thermogenic $\dot{V}O_2max$ in hypoxia in response to directional selection (4-9). In conjunction with a higher $\dot{V}O_2max$ in chronic hypoxia, high-altitude

deer mice also exhibit higher pulmonary O_2 extraction, arterial O_2 saturation, cardiac output, and tissue O_2 extraction than their lowland counterparts (9, 35). The latter is associated with several evolved changes in skeletal muscle phenotype and mitochondrial function (36-40). Highlanders have also evolved a higher Hb-O_2 affinity as a result of amino acid replacements in duplicated genes that encode the α- and β-chain subunits of the $\alpha_2\beta_2$ Hb tetramer (5, 6, 15, 20, 21, 23, 24, 26, 34, 41). We initially hypothesised that these evolved increases in Hb-O_2 affinity would be responsible for higher thermogenic capacity in highland deer mice, compared to their lowland conspecifics. To investigate the effect of genetically based changes in Hb-O_2 affinity on whole-animal performance in hypoxia, we created F_2 hybrids between high- and low-altitude deer mice (F_2 intercross breeding design) to randomize associations between allelic globin variants, and we then examined the effects of α- and β-globin variants on red blood cell P_{50} (the O_2 pressure, PO_2, at which Hb is 50% saturated), arterial O_2 saturation, thermogenic $\dot{V}O_2$max, and other physiological traits on an admixed genetic background. We performed physiological measurements before and after chronic exposure to hypoxia to test for effects of Hb genotype on trait-specific acclimation responses. We then used our empirical data in an *in silico* model of the O_2 transport pathway to examine the interactive effects of Hb-O_2 affinity and the O_2 diffusing capacity of tissues (D_TO_2) on $\dot{V}O_2$max. Our results suggest that increases in Hb-O_2 affinity only contribute to the adaptive enhancement of thermogenic $\dot{V}O_2$max in hypoxia if accompanied by a corresponding increase in D_TO_2 to augment tissue O_2 extraction.

Results

We measured thermogenic $\dot{V}O_2$max, arterial O_2 saturation, and other cardiorespiratory traits *in vivo* during acute exposure to cold heliox in normoxia (21% O_2) and hypoxia (12% O_2) in F_2 hybrid mice that possessed a diverse array of different α- and β-globin genotypes. We also performed *in vitro* measurements of red blood cell P_{50} using erythrocyte suspensions from the same set of mice. The F_2 hybrids were generated by crossing wild mice from populations at high and low altitudes to produce F_1 inter-population hybrids, followed by full-sibling matings to create 4 families of F_2 hybrid progeny with admixed genetic backgrounds. Measurements of physiological phenotypes were made before and after a 6-week acclimation period to hypobaric hypoxia (12 kPa O_2, simulating ~4,300 m above sea level). In general, hypoxia acclimation was associated with increased $\dot{V}O_2$max in hypoxia, along with increases in pulmonary ventilation, arterial O_2 saturation, heart rate, hematocrit (Hct) and blood Hb concentration ([Hb]), but also increases in red blood cell P_{50} (Figure S1, Table S1). However, hypoxia acclimation did not affect $\dot{V}O_2$max under normoxic conditions. Below, we describe the effects of Hb genotype on thermogenic $\dot{V}O_2$max and hematological traits in mice acclimated to normoxia, and then we describe how Hb genotype affects acclimation responses to chronic hypoxia.

Genetically based decreases in red blood cell P_{50} improved arterial O_2 saturation in hypoxia. In normoxia-acclimated mice, there was a significant main effect of Hb genotype on red blood cell P_{50} (P = 0.0048; Figure 1A, Table S2), which appeared to be largely attributable to the effects of α-globin variants. Mice possessing highland α-globin variants had a lower red blood cell P_{50} compared to those possessing lowland variants, reflecting a higher affinity for O_2. In contrast, Hb genotype did not affect Hct (P = 0.8339), [Hb] (P = 0.9351), or the Hill coefficient (*n*) that quantifies the cooperativity of Hb-O_2 binding (P = 0.8053; Figure S2, Table S2).

Arterial O_2 saturation varied in association with red blood cell P_{50} in hypoxia, but not in normoxia. There were significant main effects of both Hb genotype (P = 0.0189) and inspired PO_2 (P < 0.0001) on arterial O_2 saturation at $\dot{V}O_2$max, with mice exhibiting reduced saturation in hypoxia (Figure 1B, Table S2). However, the effect of inspired PO_2 on arterial O_2 saturation was influenced by genotype (genotype x PO_2 interaction, P = 0.0389), as mice with the highland α-globin genotype exhibited a smaller reduction in arterial O_2 saturation under hypoxia compared to those with the lowland genotype. Consequently, mice with highland α-globin maintained 9-14%

higher arterial O_2 saturation on average than those with lowland α-globin at hypoxic $\dot{V}O_2$max. Higher red blood cell O_2 affinity was associated with higher arterial O_2 saturation in hypoxia, as indicated by a significant negative relationship between arterial O_2 saturation and red blood cell P_{50} (P = 0.0103, R^2 = 0.2441; Figure 1C).

Genetically based variation in red blood cell P_{50} and arterial O_2 saturation had no effect on thermogenic $\dot{V}O_2$max in hypoxia. $\dot{V}O_2$max was significantly reduced in hypoxia compared to normoxia by ~24% on average (P < 0.0001; Figure 2A, Table S2). However, although Hb genotype had a significant main effect on $\dot{V}O_2$max (P = 0.0416), $\dot{V}O_2$max in hypoxia did not follow the pattern of variation seen for arterial O_2 saturation. As such, hypoxic $\dot{V}O_2$max was not correlated with arterial O_2 saturation in hypoxia (Figure 2B). Instead, the observed variation in $\dot{V}O_2$max appeared to be associated with variation in heart rate, which was also significantly affected by inspired PO_2 (P < 0.0001), though the effect of genotype was only marginally significant (P = 0.0545; Figure S3A, Table S2). Total ventilation, tidal volume, and breathing frequency were was unaffected by Hb genotype (Figure S3, Table S2).

Hb genotype influenced the acclimation responses of red blood cell P_{50} and arterial O_2 saturation to chronic hypoxia. There were main effects of hypoxia acclimation that tended to increase both red blood cell P_{50} (P = 0.0002) and arterial O_2 saturation measured at $\dot{V}O_2$max in hypoxia (P = 0.0005), but the acclimation response appeared to differ between genotypes (Figure 3, Table S3). Mice with the lowland α-globin variant exhibited no plasticity in red blood cell P_{50} in response to hypoxia acclimation, whereas mice with highland α-globin increased red blood cell P_{50} to values that were comparable to mice with lowland α-globin. Conversely, mice with lowland α-globin showed much greater plasticity in arterial O_2 saturation in hypoxia following hypoxia acclimation, with all individuals increasing saturation (on average by ~13% saturation units). Mice with highland α-globin showed little to no change in saturation after hypoxia acclimation. Hypoxia acclimation increased Hct (P < 0.0001) and [Hb] (P < 0.0001; Figure S1), but neither these traits nor the Hill coefficient were influenced by Hb genotype (Figure S2, Table S3).

$\dot{V}O_2$max in hypoxia increased after hypoxia acclimation (P = 0.0013), but this response was not influenced by Hb genotype (P = 0.1764; Figure S4, Table S3). The magnitude of change in hypoxic $\dot{V}O_2$max following hypoxia acclimation was not associated with the magnitude of change in arterial O_2 saturation (Figure 3C). Hypoxia acclimation also increased heart rate (P = 0.0031), total ventilation, (P < 0.0001), tidal volume (P = 0.0005), and breathing frequency (P < 0.0001) measured at $\dot{V}O_2$max in hypoxia, but none of these traits were affected by Hb genotype (Figure S5, Table S3). Normoxic $\dot{V}O_2$max was not affected by hypoxia acclimation or Hb genotype, nor were the measurements of heart rate, total ventilation, tidal volume, or breathing frequency at normoxic $\dot{V}O_2$max affected by Hb genotype (Figure S6, Table S4). However, there was a main effect of genotype on arterial O_2 saturation measured at $\dot{V}O_2$max in normoxia (P = 0.0291) that appeared to result from slightly lower saturation values in mice with characteristic lowland α- and β-globin genotypes ($\alpha^{LL}\beta^{LH}$; Figure S6B, Table S4).

Sensitivity analysis suggested that effects of Hb-O_2 affinity on $\dot{V}O_2$max in hypoxia are contingent on tissue O_2 diffusing capacity (D_TO_2). We examined the interactive effects of Hb-O_2 affinity and D_TO_2 on $\dot{V}O_2$max in hypoxia using a mathematical model of O_2 flux through the O_2 transport pathway. We generated the initial solutions of the model using empirical data collected for deer mice, and then performed a sensitivity analysis to determine the effects of increasing D_TO_2 on $\dot{V}O_2$max at each of the red blood cell P_{50} values for mice with characteristic highland ($\alpha^{HH}\beta^{HH}$) and lowland ($\alpha^{LL}\beta^{LH}$) Hb genotypes. Increasing D_TO_2 by 50% increased $\dot{V}O_2$max, but the effect was greater with the P_{50} of the high-affinity $\alpha^{HH}\beta^{HH}$ genotype (10.7%) than for the lower affinity $\alpha^{LL}\beta^{LH}$ genotype (7.7%; Figure 5A). The effect of P_{50} was accentuated when D_TO_2 was increased above 37%, when venous PO_2 (and thus venous O_2 saturation) fell to zero at the higher P_{50} (Figure 5B). These results indicate that an increase in Hb-O_2 affinity only contributes to

an enhancement of $\dot{V}O_2$max in hypoxia if it is paired with an increase in D_TO_2 in thermogenic tissues (i.e., skeletal muscle and/or brown adipose tissue).

Discussion

Our study provides evidence that the adaptive benefit of increasing Hb-O_2 affinity is contingent on the capacity of active tissues to extract O_2 from the blood. In agreement with previous studies (5, 6, 20, 21, 24, 26, 42), our data from F_2 inter-population hybrids demonstrate that Hb variants from high-altitude deer mice confer a higher Hb-O_2 affinity than Hb from lowland conspecifics, and that this evolved increase in affinity augments arterial O_2 saturation in hypoxia by 9-14%. However, these genetically based changes alone did not augment $\dot{V}O_2$max (*i.e.*, aerobic performance) in hypoxia. Modeling of the O_2 transport pathway revealed that increases in Hb-O_2 affinity would only be expected to enhance $\dot{V}O_2$max in hypoxia if O_2 diffusing capacity were increased to augment tissue O_2 extraction. Importantly, recent evidence suggests that high-altitude mice do possess an enhanced capacity for O_2 diffusion through the evolution of a highly aerobic skeletal muscle phenotype (36-40). This involves greater capillary density, higher proportional abundance of oxidative fibre types, increased volume density of subsarcolemmal mitochondria, and augmented mitochondrial respiratory capacities. Our results therefore suggest that increases in both Hb-O_2 affinity and tissue O_2 diffusing capacity likely contributed to the adaptive increases in $\dot{V}O_2$max in high-altitude deer mice. These findings suggest the testable hypothesis that other hypoxia-adapted, high-altitude vertebrates that have evolved derived increases in Hb-O_2 affinity will also have evolved increases in tissue capillarity and/or other changes that augment O_2 diffusing capacity.

The genetically based differences in Hb function led to predictable differences in arterial O_2 saturation during acute and chronic hypoxia. Amino acid variation in Hb genes is not always associated with changes in O_2-binding properties (16, 43), and even in cases where it has been possible to document causal effects of specific mutations on Hb function (13, 15, 23-26, 34, 41, 44-47), the *in vivo* effects on blood oxygenation have rarely been examined. Our study suggests that it is critically important to examine how genetic changes in proximal biochemical phenotypes affect higher-level physiological phenotypes (*e.g.*, arterial O_2 saturation and $\dot{V}O_2$max in hypoxia) to fully understand their potential adaptive significance.

Genetic variation in Hb altered the acclimation response to chronic hypoxia, as highland α-globin genotypes were associated with increased plasticity in Hb-O_2 affinity of red blood cells. This variation was likely a result of differences in sensitivity to 2,3-diphosphoglycerate (2,3-DPG), an allosteric modulator of Hb-O_2 affinity. Concentrations of 2,3-DPG in erythrocytes are known to increase in response in chronic hypoxia, which tends to reduce red blood cell Hb-O_2 affinity (48-52). Previous studies have shown that Hb from high-altitude deer mice are more sensitive to 2,3-DPG in the presence of Cl^- than Hb from low-altitude mice (24, 26). Therefore, in the current study, if red blood cell concentrations of 2,3-DPG were comparable across genotypes, differences in 2,3-DPG sensitivity could explain the differences in plasticity of red blood cell Hb-O_2 affinity. This mechanism may also explain why genotypes differed in the magnitude of plasticity in arterial O_2 saturation in response to chronic hypoxia. Several physiological adjustments contribute to increasing arterial O_2 saturation after hypoxia acclimation, including increases in total ventilation (Figure S1) and adjustments in lung function to augment pulmonary O_2 diffusion (35, 53), and these effects could potentially be counteracted by reductions in red blood cell Hb-O_2 affinity. Such reductions in affinity did not occur in mice with lowland α-globin, such that they experienced greater improvements in arterial O_2 saturation after hypoxia acclimation.

Our results indicate that the adaptive benefit of increasing Hb-O_2 affinity is contingent on the O_2 diffusing capacity of active tissues. Our study provides empirical evidence that genetically based

increases in Hb-O_2 affinity and arterial O_2 saturation alone are not sufficient to improve aerobic capacity in hypoxia. We also demonstrate that the adaptive benefit of increasing Hb-O_2 affinity is contingent on having a tissue O_2 conductance (D_TO_2) that is sufficiently high to take advantage of the greater arterial O_2 saturation and extract more O_2 from the blood. The relationship between Hb-O_2 affinity and $\dot{V}O_2$max in hypoxia is a contentious topic (12, 25, 28), with different empirical studies and theoretical models providing contradictory results (5, 27, 29, 30, 33, 54). In fact, previous investigation in deer mice has shown that mice possessing highland α-globin alleles with higher Hb-O_2 affinity did have higher $\dot{V}O_2$max in hypoxia than mice with lowland α-globin haplotypes (5). However, in this previous study (in which genotyping was based on protein electrophoresis), different α-globin alleles were backcrossed into a highland genetic background (5), unlike the current study in which alternative allelic variants were randomized against an admixed highland/lowland background. As discussed above, highland deer mice appear to have evolved a higher capacity for O_2 diffusion and utilization in skeletal muscles than their lowland conspecifics. It is therefore possible that the highland mice used in this previous study (5) had a higher D_TO_2 than the F_2 inter-population hybrids used in our present study, which would explain the observed differences in the relative influence of Hb genotype on $\dot{V}O_2$max. Indeed, our modeling shows that the adaptive benefits of increasing Hb-O_2 affinity are critically dependent on D_TO_2. Together, our findings suggest adaptive increases in $\dot{V}O_2$max in high-altitude deer mice may have been facilitated by evolved increases in D_TO_2, which were required in order for increases in Hb-O_2 affinity to confer an adaptive benefit at high-altitude.

Materials and Methods

Animals and acclimation treatments. Wild deer mice (*Peromyscus maniculatus*) were live-trapped at high altitude (Mount Evans, CO, USA; 4350 m above sea level) and low altitude (Nine Mile Prairie, NE, USA; 430 m above sea level), from which one family of first-generation inter-population hybrids (F_1) was created by crossing a highland male and a lowland female. These F_1 hybrids were raised to maturity and used for full-sibling matings to produce 4 families of second-generation hybrid progeny (F_2). Each F_2 mouse was genotyped to determine the sequence of its α- and β-globin haplotypes, resulting in the 5 distinct combinations of highland and lowland haplotypes of α- and β-globin that were studied here. Mice were raised to adulthood in standard holding conditions in normoxia, and the physiological measurements described below were then conducted before and after acclimation to hypobaric hypoxia (12 kPa O_2) for 6 weeks. All animal protocols were approved by institutional animal research ethics boards. Additional details on holding conditions, genotyping, and acclimations can be found in the Supplementary Methods (electronic supplementary material).

Respirometry and pulse oximetry. We measured thermogenic $\dot{V}O_2$max concurrent with measurements of arterial O_2 saturation, heart rate, and pulmonary ventilation. Thermogenic $\dot{V}O_2$max was measured by open-flow respirometry as the highest O_2 consumption rate over 10 s during a 10 min exposure to acute cold (-5˚C) in heliox. These measurements were made in both normoxia (21% O_2, 79% He) and hypoxia (12% O_2, 88% He), both before and after hypoxia acclimation, while simultaneously measuring arterial O_2 saturation and heart rate by pulse oximetry, as well as ventilation by plethysmography. Detailed methods have been previously reported (9, 35) and can be found in the Supplementary Methods.

Hematology. Hematology was measured both before and after hypoxia acclimation. Blood samples were taken from the facial vein, 3 days after $\dot{V}O_2$max measurements. We measured Hb content using Drabkin's reagent (according to instructions from the manufacturer, Sigma-Aldrich) and hematocrit by spinning the blood in capillary tubes at 12,700 *g* for 5 min. The O_2 affinity of intact erythrocytes was measured using 10 μl blood in 5 ml buffer containing 0.1 M Hepes, 0.05 M EDTA, 0.1 M NaCl, 0.1% bovine serum albumin, and 0.2% antifoaming agent at pH 7.4.

Oxygen dissociation curves were generated at 37 °C using a Hemox Analyzer (TCS Scientific), and red blood cell P_{50} and Hill coefficient (n) were calculated using Hemox Analytic Software.

Statistics. We used linear mixed effects models to test for the effects of Hb genotype and acclimation condition using the lme4 (55) package in R (v.3.1.3, R Core Team, 2013). We carried out one set of models to examine the fixed effects of Hb genotype in normoxia-acclimated mice, in absence of the effects of hypoxia acclimation, and with inspired PO_2 as an additional fixed effect. We then carried out a second set of models including data from both before and after chronic hypoxia exposure to examine the effects of Hb genotype, hypoxia acclimation, and their interaction. We used a backwards model selection approach, in which initial models included sex, family, and individual subject as random factors, as well as body mass as a covariate. If these terms had P values above 0.1, they were removed by stepwise backward deletion (starting with the term with the highest P value) and the model was re-run until all terms in the model (with the exception of fixed factors and individual subject) had P values below 0.1. Family was thus included in only 6 of the models (see Tables S1-S4), while the effects of sex were never significant and were removed from all models. Tukey's HSD *post hoc* tests were performed to test for pairwise differences between genotypes within an acclimation/PO_2 treatment, and between acclimation/PO_2 treatment groups within each genotype. Data are presented as individual values and as mean ± SEM, unless otherwise stated.

Modeling the O_2 transport pathway. We investigated the combined effects of changes in red blood cell P_{50} and tissue oxygen diffusing capacity (D_TO_2) on $\dot{V}O_2$max using a mathematical model of the oxygen transport pathway. This model was built using previously established equations, as described in detail in the Supplementary Methods (30, 56-59). We then conducted sensitivity analyses of the effects of increasing D_TO_2 on $\dot{V}O_2$max using the ancestral 'lowland' Hb P_{50} of $\alpha^{LL}\beta^{LH}$ mice versus the 'highland' Hb P_{50} of $\alpha^{HH}\beta^{HH}$ mice. $\dot{V}O_2$max and D_TO_2 values are expressed relative to the 'ancestral values' from the initial solution using lowland P_{50}.

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Figures and Tables

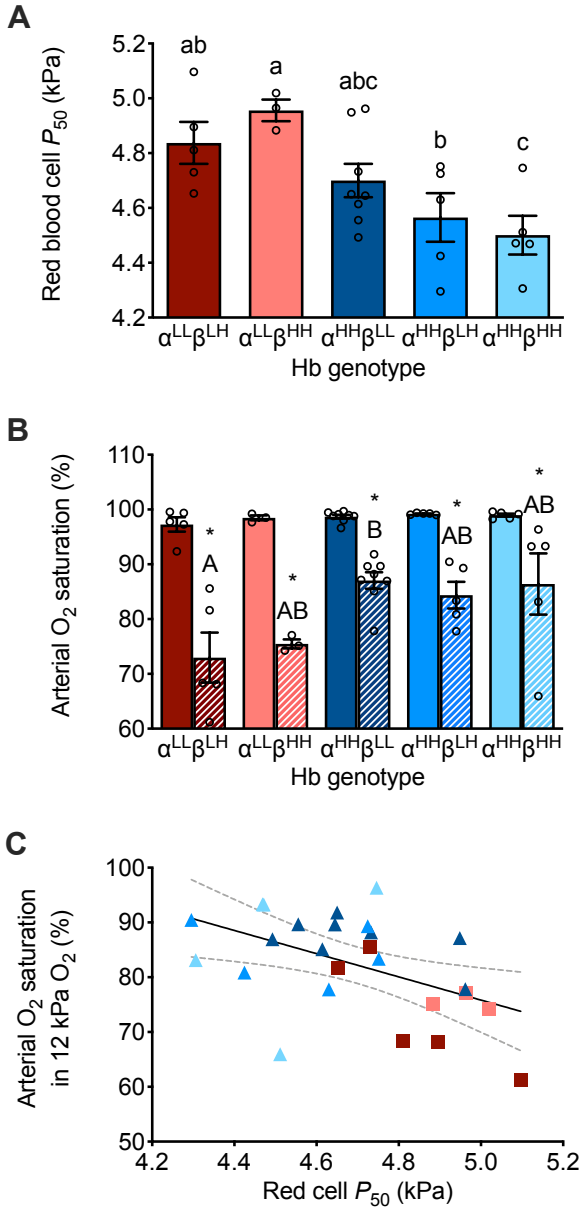

Figure 1. Variation in red blood cell O_2 affinity and arterial O_2 saturation associated with hemoglobin (Hb) genotype in F_2 inter-population hybrid deer mice acclimated to normoxia. **A**) Red blood cell P_{50} (O_2 pressure at 50% saturation). **B**) Arterial O_2 saturation at $\dot{V}O_2$max measured in normoxia (21 kPa O_2; solid bars) and hypoxia (12 kPa O_2; hatched bars). Bars display mean ± SEM (n = 3-8) with individual data superimposed (circles). Different α- and β-globin genotypes are shown as superscripts with 'L' representing the lowland haplotype and 'H' representing the highland haplotype. *P < 0.05, hypoxia vs. normoxia within a genotype. P < 0.05 between genotypes for values not sharing a letter. **C**) Linear regression of arterial O_2 saturation in hypoxia and red blood cell P_{50} for individual data (P = 0.0103, R^2 = 0.2441; dotted line represents 95% confidence interval).

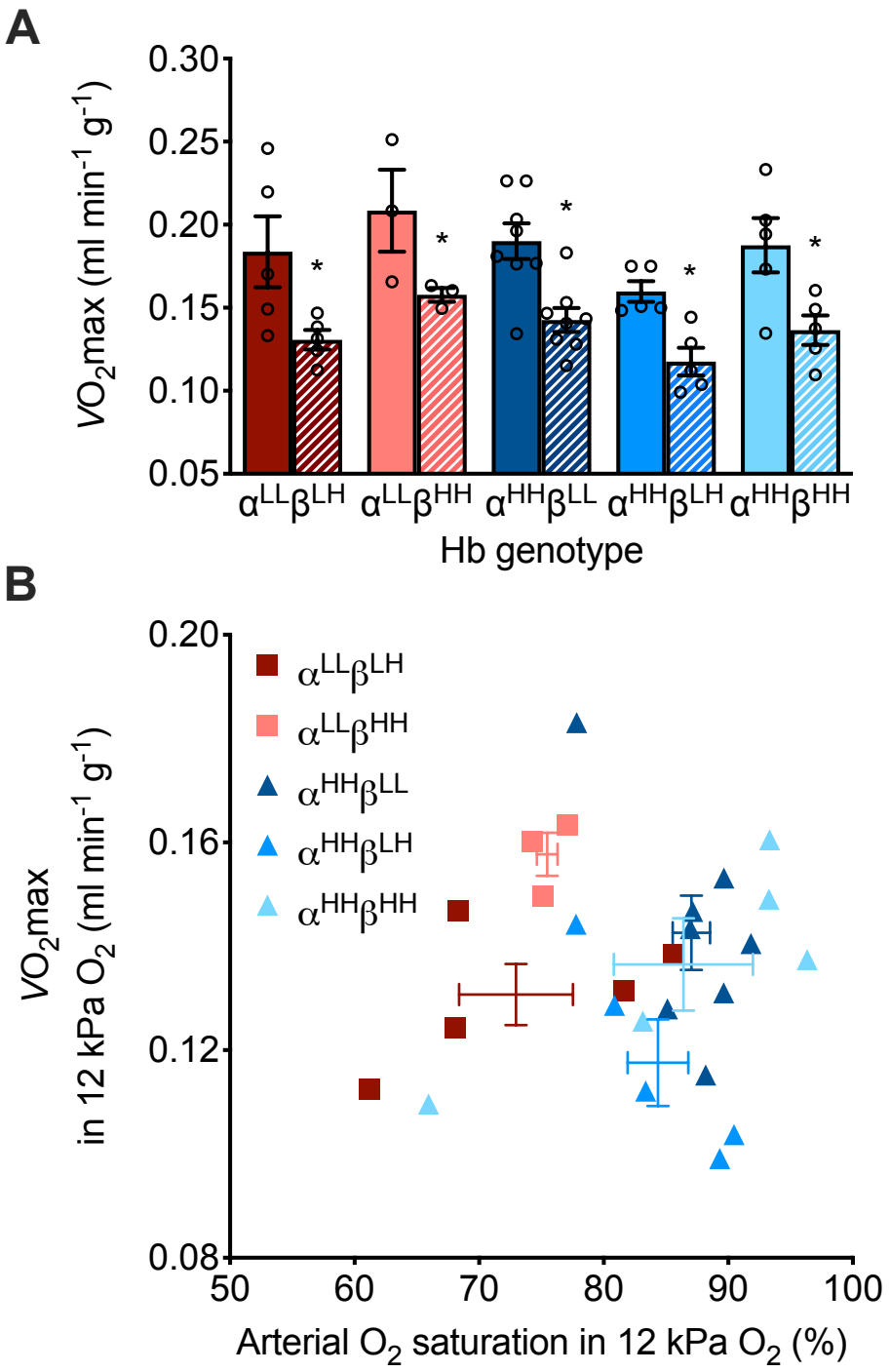

Figure 2. Variation in $\dot{V}O_2$max was unrelated to variation in arterial O_2 saturation in F_2 inter-population hybrid deer mice acclimated to normoxia. **A**) $\dot{V}O_2$max measured in normoxia (solid bars) and hypoxia (hatched bars). See Figure 1 for details on hemoglobin (Hb) genotypes and symbols. **B**) There was no correlation between hypoxic $\dot{V}O_2$max and arterial O_2 saturation in hypoxia (P=0.8103) across individuals (mean ± SEM for each genotype are shown as error bars).

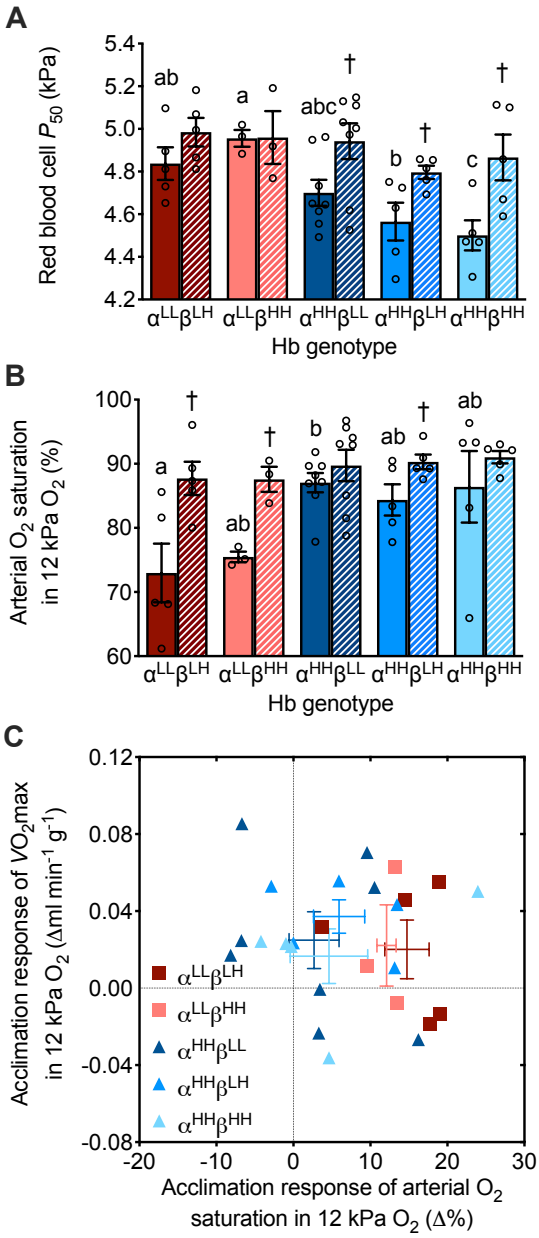

Figure 3. The effects of hypoxia acclimation on red blood cell O_2 affinity and arterial O_2 saturation differed between hemoglobin (Hb) genotypes in F_2 inter-population hybrid deer mice, but the effects of hypoxia acclimation on $\dot{V}O_2$max did not. **A**) Red blood cell P_{50} (O_2 pressure at which Hb is 50% saturated) and **B**) arterial O_2 saturation at $\dot{V}O_2$max in hypoxia, measured before (solid bars) and after (hatched bars) 6-week acclimation to hypobaric hypoxia (12 kPa O_2). $^{\dagger}P < 0.05$ vs. pre-acclimation value within a genotype. $P < 0.05$ between genotypes within an acclimation condition for values not sharing a letter. **C**) The change in hypoxic $\dot{V}O_2$max plotted against the change in arterial O_2 saturation in hypoxia in individuals in response to hypoxia acclimation (mean ± SEM for each genotype are shown as error bars). See Figure 1 for other details on Hb genotypes.

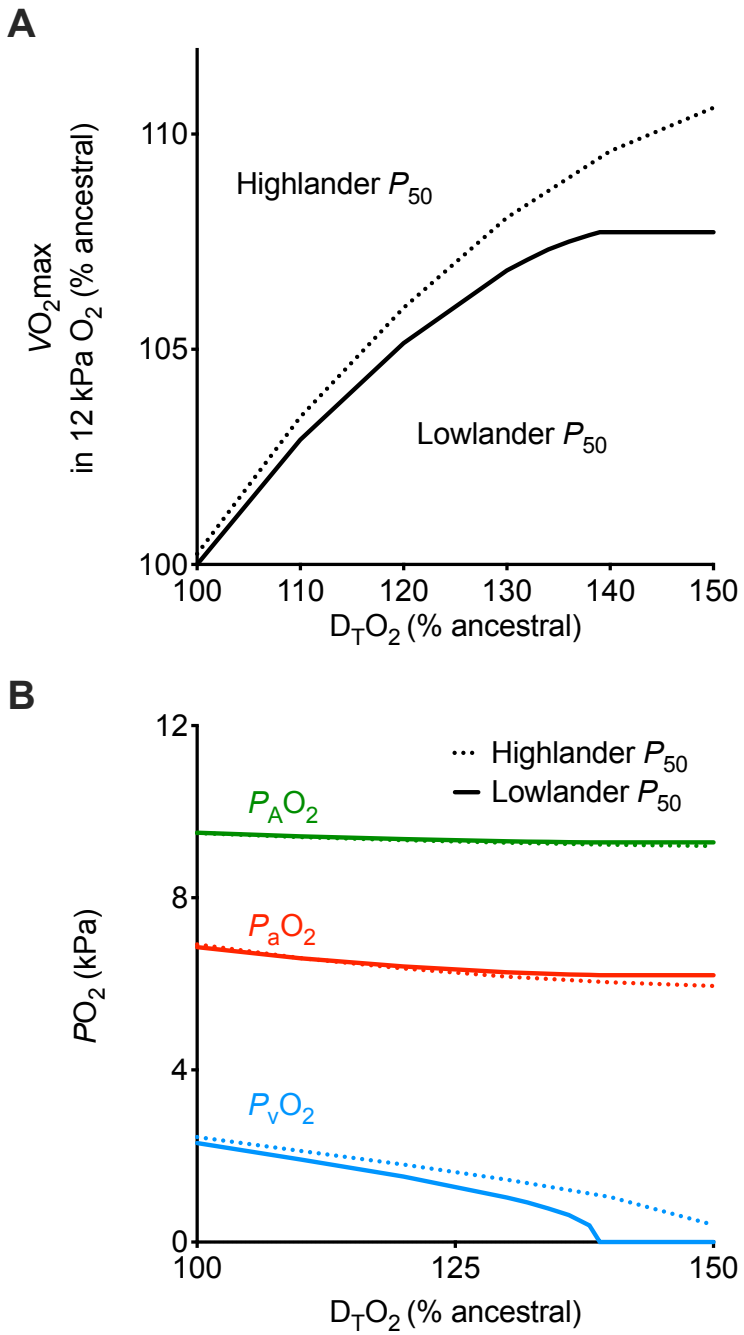

Figure 4. Effects of increasing tissue O_2 diffusing capacity (D_TO_2) on hypoxic $\dot{V}O_2$max and O_2 partial pressures (PO_2) using mathematical modeling of the O_2 transport pathway. **A**) Relative changes in hypoxic $\dot{V}O_2$max and **B**) changes in alveolar (P_AO_2), arterial (P_aO_2) and venous (P_vO_2) PO_2 in response to relative increases in D_TO_2. Effects were modeled using the red blood cell P_{50} of mice with hemoglobin genotypes that were most characteristic of lowlanders ($\alpha^{LL}\beta^{LH}$) and highlanders ($\alpha^{HH}\beta^{HH}$).