Pulmonary Arteriolar Vasoconstriction Dominates the Acute Pulmonary Vascular Response to Hypoxia

Oxygen tension is a major regulator of pulmonary vascular tone and a physiological mechanism for matching perfusion with ventilation. A fall in alveolar $P_{O_2}$ is the main stimulus for HPV, but a reduction in mixed venous and bronchial arterial $P_{O_2}$ may also contribute. Ventilation of intact lungs with a hypoxic gaseous mixture (eg, fraction of inspired oxygen=0.10) leads to acute pulmonary vasoconstriction throughout the pulmonary vascular bed, including nonmuscular arterioles, capillaries, and veins, but is most pronounced in small pulmonary arterioles. That said, HPV is not distributed evenly throughout the lung and lung perfusion is inhomogeneous during hypoxia.

HPV has at least 2 phases (Figure 1A). An initial constrictor response that starts within seconds and reaches a maximum within minutes is followed by a sustained phase, which develops after 30 to 120 minutes. A transient phase of vasodilation may be observed linking the two, and a third phase of even more pronounced vasoconstriction can occur after 120 minutes. The phases are regulated, at least in part, by different signaling pathways. In vivo, factors such as neurohumoral mediators, red blood cells, and lung innervation may influence the response.

Alveolar capillaries have been proposed as a site for oxygen sensing with propagation of the hypoxic signal by endothelial membrane depolarization to upstream arterioles in a connexin 40-dependent manner. However, the recapitulation of contraction to hypoxia in cultured pulmonary artery smooth muscle cells, the effector cells, indicates that an oxygen-sensing mechanism is intrinsic to these cells. Both mitochondria and nicotinamide adenine dinucleotide (phosphate) oxidases have been suggested as oxygen sensors. A change in the levels of reactive oxygen species is thought to be important, but there is a lack of agreement regarding whether the signal is an increase or decrease in reactive oxygen species (Figure 2).

Differences in techniques used contribute to the different observations, but the spatial distribution of reactive oxygen species signaling may also be significant.

The second phase of HPV is influenced by endothelial cell function. The endothelium releases a variety of vasoactive mediators, such as endothelin 1, prostanoids, and nitric oxide (NO; Figure 3), and their production is perturbed by hypoxia. For example, oxygen is a substrate for NO

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In addition, sustained HPV has been shown to depend on glucose level.24 Elevated PAP in a telemetered rat takes days to return to baseline on return to normoxia in isolated perfused rabbit lungs, even if the perfusate is replaced to remove hypoxia-stimulated vascular remodeling of pulmonary vessels. This has been described as being outward rather than inward) and the extent of vascular obstruction to blood flow (ie, the argument being that vascular remodeling contributes to and sustains HPV, underlying the importance of the RhoA/Rho kinase signaling pathway in regulating pulmonary vascular tone. Acute administration of a Rho kinase inhibitor significantly reduces pulmonary vascular resistance in chronically hypoxic rats,26 advancing the argument that vasoconstriction is an important pathophysiological mechanism in high-altitude pulmonary hypertension (HAPH), perhaps as important or more important than vascular remodeling.27

**Cardiac Response to Chronic Hypoxia**

Studies of healthy subjects exposed to hypoxia report an increase in resting heart rate and an initial increase in cardiac output in an attempt to maintain oxygen delivery to tissues.47 After 2 to 3 days of hypoxia, stroke volume falls. Heart rate remains elevated, and so cardiac output remains at or just below sea level. Oxygen delivery to tissues is maintained by increased oxygen extraction. The roles of activation of the sympathetic nervous system, hypovolemia (from hyperventilation and increased diuresis), hypocapnia (from hyperventilation), and myocardial contractility in this response are difficult to discern.47 On the whole, myocardial contractility is preserved, although reversible reductions in cardiac mass and myocardial phosphocreatine/ATP have been documented in healthy volunteers after a 17-day trek to 5300 m.48 Right heart failure is a risk in some previously healthy individuals, precipitated by more extreme pulmonary

**Vascular Remodeling Contributes to and Sustains the Chronic Pulmonary Vascular Response to Hypoxia**

Chronic global alveolar hypoxia is accompanied by structural remodeling of pulmonary vessels. This has been described in a number of species, including rat,26 cow,29 and humans,30 although some species seem resistant.31 All of the layers of the vascular wall, including fibroblasts, are involved in the remodeling (Figure 4),32,33 but the hallmark of the vascular response to chronic hypoxia is increased muscularization of distal vessels with extension of muscle into previously unmuscularized arterioles.28,30

Hypoxia leads to changes in endothelial cell membrane properties that compromise barrier function, resulting in an influx of plasma proteins that may activate vascular wall proteases.34 In addition, mechanical stress, blood-borne and locally produced factors, and the recruitment of circulating cells act collectively to drive the vascular remodeling of small and large pulmonary vessels, with increasing recognition of the role of inflammation (Figure 4).35–37 Rapid expansion of the adventitial vasa vasorum serves to facilitate the arrival of these cells.33

HIFs and nuclear factor-xB are key transcriptional regulators of the proliferative and inflammatory responses to hypoxia. Pulmonary hypertension in hypoxic mice haploinsufficient for HIF-1α (Hif1α+/-) or HIF-2α (Hif2α+/-) is attenuated.38,39 Conversely, gain-of-function mutations in HIF-2α are associated with the development of pulmonary hypertension in mice and humans.40–42

Although in part caused by and adaptive to the increase in hemodynamic stress, the vascular remodeling contributes to and sustains the elevated PAP. Indeed, the structural changes take a considerable time to resolve on return to a sea-level oxygen environment and may persist in some form.43 The extent to which the structural changes in pulmonary resistance vessels infringe on the lumen and contribute a physical obstruction to blood flow (ie, the argument being that vascular growth is outward rather than inward) and the extent of vascular rarefaction in response to chronic hypoxia are unclear.44 These may vary between species.

Mechanisms other than narrowing of the vessel lumen are relevant to this discussion, specifically the contribution of changes in vascular compliance.45 Changes in the stiffness of proximal vessels leads to changes in the propagation of high-energy pulsatile waves. These are transmitted to the microcirculation, where they might perpetuate or potentially even cause the microcirculatory changes, as well as contribute to the burden on the RV.46

**Figure 1.** Contribution of hypoxic pulmonary vasoconstriction (HPV) and vascular remodeling to the rise in pulmonary artery pressure (PAP) in chronic hypoxia. A. The initial rise in PAP in hypoxia is driven by HPV. The pressor response to hypoxia does not return to baseline on return to normoxia in isolated perfused rabbit lungs, even if the perfusate is replaced to remove hypoxia-stimulated vascular remodeling after removal from 2 weeks in a hypoxic chamber. B. Elevated PAP in a telemetered rat takes days to return to baseline.

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**Table 1.**

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**Table 2.**

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**Figure 2.**

**Figure 3.**

**Figure 4.**

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**Figure 5.**

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**Figure 6.**

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**Figure 7.**

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vascular responses to hypoxia, and also in the context of chronic mountain sickness (CMS), from pronounced erythrocytosis and fluid retention (from relatively higher \( Pco_2 \)).

**Exercise**

Exercise capacity is reduced at altitude, even after acclimatization, but the contribution of pulmonary hypertension is controversial.\(^5^9,6^0\) PAP increases more sharply with the increase in cardiac output on exercise at altitude than at sea level.\(^5^1\) This augmented rise in PAP with exercise can persist for some time in acclimatized highlanders on descent to sea level, most likely reflecting structural remodeling of the pulmonary vasculature with chronic exposure.\(^5^4\) The increase in PAP may impair gas exchange from interstitial and alveolar edema and reduce maximal cardiac output, leading to a reduction in oxygen transport to exercising muscles.\(^4^9\) However, definitive data from direct measurements of RV function at altitude are few, and not all are convinced that the improvement in exercise capacity at altitude reported with some pulmonary vasodilators is attributed to a reduction in RV afterload.\(^5^0\)

**Erythropoietic Response to Hypoxia**

Exposure to hypoxia leads to changes in blood-O\(_2\) affinity and stimulates red cell production in an attempt to improve tissue oxygenation. Increased red cell 2,3 diphosphoglycerate levels have an allosteric effect on hemoglobin, reducing its affinity for O\(_2\) and facilitating its release to tissues, although this is at the expense of impairing O\(_2\) capture as blood passes through the lungs. Increasing red cell mass also has its downside, as it increases blood viscosity. Little attention has been paid to the contribution of increased blood viscosity to PAP, because the increase in PAP precedes the rise in hemoglobin, and patients

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**Figure 2.** Signaling mechanisms underlying acute hypoxic pulmonary vasoconstriction (HPV). Pathways activated by hypoxia are depicted in blue; those inhibited by hypoxia are depicted in red. Both mitochondria and nicotinamide adenine dinucleotide (phosphate) oxidases have been suggested as oxygen sensors. A reduction in the cytosolic redox state could inhibit voltage-dependent potassium channels, subsequent membrane depolarization of PASMCs, opening of \(\lambda\)-type calcium channels and Ca\(^{2+}\) influx.\(^2^0\) By contrast, increased cytosolic ROS levels can result in Ca\(^{2+}\) release from the SR, possibly through the oxidation of cysteine residues in RyRs and the opening of IP\(_3\)-gated calcium stores.\(^5^9\) Increased ROS could also provoke an influx of extracellular Ca\(^{2+}\) or Na\(^+\) through transient receptor potential channels (TRPC6).\(^2^1\) In this scenario, the increase of acute hypoxia-induced ROS triggers an accumulation of DAG, resulting from the activation of phospholipase C or phospholipase D or inhibition of DAG-degrading DAG kinases. Another proposal assumes that acute hypoxia leads to inhibition of the respiratory chain and a subtle decrease in ATP production, which does not affect energy state, but rather acts as a mediator and alters the cellular AMP/ATP ratio. An increase in the AMP/ATP ratio activates AMPK, followed by an increase in cADPR that triggers the release of [Ca\(^{2+}\)] through RyR of SR.\(^3^\) The level of ROS could be relevant through ROS-dependent alteration of function of AMPK and cADPR. AMPK indicates AMP-activated protein kinase; Ca\(^{2+}\), calcium; cADPR, cyclic ADP-ribose; DAG, diacylglycerol; DGK, DAG kinases; Em, membrane potential; GSH, glutathione; GSSG, glutathione disulfide; IP\(_3\), inositol trisphosphate; IP\(_3\)R, inositol trisphosphate receptor; K\(^+\), potassium; K\(_v\), voltage-dependent potassium channels; Na\(^+\), sodium; NAD\(^+\), nicotinamide adenine dinucleotide; NADH, reduced form of NAD; NAD(P)H, nicotinamide adenine dinucleotide (phosphate) oxidase; O\(_2\), oxygen; PASMC, pulmonary arterial smooth muscle cell; ROS, reactive oxygen species; RyR, ryanodine receptors; SR, sarcoplasmic reticulum; TRPC6, transient receptor potential cation channel 6; and VOCC, voltage–dependent calcium channels (includes \(\lambda\)-type calcium channels).
changes in PASMC myofilament sensitivity to Ca\textsuperscript{2+}, arising from signaling, also contributes to sustained HVP. ADMA indicates (PKC), or a decreased activation of MLCP by decreased NO inhibition of MLCP via RhoA/Rho kinase or protein kinase C.

5-fold among individuals. Extreme of HPV can vary and within species, and in humans the magnitude of HPV varies according to altitude and ethnic background, but some evidence is variously recorded, depending on the subject population.

The rise in PAP in chronic hypoxia is generally modest, certainly compared with that seen in idiopathic pulmonary arterial hypertension, and is compatible with a normal life at high altitude. However, variation in the pulmonary vascular response to hypoxia is well recognized, both between and within species, and in humans the magnitude of HPV can vary =5-fold among individuals. Extreme responders are at high risk of presenting acutely on arrival at altitude with high-altitude pulmonary edema (HAPE) or over weeks, months, and years with right heart failure secondary to severe pulmonary hypertension or excessive erythrocytosis.

**High-Altitude Pulmonary Edema**

The pathognomonic clinical feature is breathlessness accompanied by cough, initially dry but later productive of white and then pink frothy sputum. Tachycardia, mild pyrexia, and sometimes cyanosis are also evident. The chest radiograph shows pulmonary edema. The condition develops in susceptible individuals within the first 2 to 4 days of arrival at altitudes above 2500 m. It is precipitated by rapid ascent and exercise and is potentially lethal if not treated. The incidence is variously recorded, depending on the subject population, rapidity of ascent, and final altitude; everyone is at risk of HAPE if they ascend fast and high enough. It is caused by exaggerated and uneven HPV leading to high capillary pressures in regions of the lung and an exudation that might invoke a secondary inflammatory response. Susceptible individuals exhibit a marked rise in PAP on exposure to hypoxia mediated by pulmonary arterial vasoconstriction and a greater rise in PAP on exercise in a normal oxygen environment, indicating a hyperactive pulmonary circulation.

The emphasis in management is on prevention. Travelers should manage their rate of ascent to 300 to 500 m per day along with a day of rest every 3 to 4 days when traveling above 3000 m. Pharmacologic measures that have been evaluated and demonstrated efficacy in reducing the incidence of HAPE include slow-release nifedipine (30 mg BID), the phosphodiesterase type 5 inhibitor tadalafil (10 mg BID), and dexamethasone (8 mg BID). The \( \beta_2 \)-agonist salmeterol (125 \( \mu \)g BID) by inhalation appears to be less effective.

When treatment is required, consideration should be given to descent to a lower altitude coupled with supplemental oxygen (2–4 L/min) where possible. Nifedpine is the standard treatment. A phosphodiesterase type 5 inhibitor may be helpful but has not been formally trialed. There is no role for diuretics. A fully conscious person with mild-to-moderate HAPE may be managed at altitude if the appropriate expertise and facilities are available.

**HAPH and Heart Failure**

By convention, the definition of HAPH is a resting mean PAP >30 mm Hg. This needs revisiting and reconciling with international guidelines for the definition of pulmonary hypertension, which is a resting mean PAP >25 mm Hg.

Data on the prevalence of HAPH are sparse. Prevalence will vary according to altitude and ethnic background, but some 14% of Kyrgyz highlanders have been found to have ECG evidence of right ventricular hypertrophy. A much smaller percentage progress to and present with heart failure.

The RV generally copes well with a pressure load, and there is doubt as to whether pressure load itself is sufficient to cause heart failure, suggesting that other factors, such as myocardial hypoxia and neurohumoral factors, are important. Nonetheless, pulmonary hypertension progressing to fatal right heart failure, recognized as infantile subacute mountain
sickness, has been described in infants of Chinese Han origin who are born at low altitude and taken to high altitudes. They develop heart failure within a few weeks or months and the pathology reveals extreme medial hypertrophy of the small pulmonary arteries accompanied by hypertrophy and dilation of the RV. Heart failure has also been described in Indian soldiers posted at the high-altitude borders in China and occasionally in previously healthy travelers, and HAPH is thought to be the major factor.

Descent to lower altitude is life saving for severe cases of heart failure. There are a number of potential pharmacologic treatments for managing less severe disease, but few have been formally trialed in HAPH. Phosphodiesterase type 5 inhibitors appear effective at reducing pulmonary vascular resistance, and acetazolamide and the Rho-kinase inhibitor, fasudil, are promising. The benefits of endothelin receptor antagonists are less clear.

Chronic Mountain Sickness

The defining feature of chronic mountain sickness (CMS) is excessive erythrocytosis accompanied by neurologic symptoms, such as headache, dizziness, and fatigue. Evidence for epigenetic regulation of pulmonary vascular remodeling comes from experiments with HDACs. Class I HDAC inhibitors markedly decreased cytokine/chemokine mRNA expression levels in these cells proliferating adventitial fibroblasts and their ability to induce monocyte migration and inflammation. This in part may be attributed to modulation of microRNA-124 expression. ECAM indicates endothelial cell adhesion molecule; FGF, fibroblast growth factor; HDAC, histone deacetylase; GM-CSF, granulocyte macrophage colony stimulating factor; HIF, hypoxia-inducible factor; ICAM, intercellular adhesion molecule; IL-1β, interleukin 1β; IL-6, interleukin 6; NO-sGC-cGMP, nitric oxide-soluble guanylate cyclase-cyclic GMP; PDGF, platelet-derived growth factor; PGI₂, prostacyclin; ROS, reactive oxygen species; TRPC6, transient receptor potential cation channel 6; and VCAM, vascular cell adhesion molecule.
vascular resistance was also reduced and cardiac output increased without a change in pulmonary pressure.

**Insights From Humans Adapted to Hypoxia**

**Variation in Susceptibility to High-Altitude Pulmonary Vascular Disease**

The variation in pulmonary vascular response has a strong genetic basis, which provides the substrate for environmental selection pressures and adaptation to high-altitude living. Tibetans appear less susceptible than recent migrants to HAPH\(^{77,78}\) and CMS,\(^{79}\) most likely the result of living above 3000 m for thousands of years. Data are few, but PAP measurements in ethnic Tibetans living over 3600 m are in the range typical of healthy adults at sea level,\(^{77,78}\) and postmortem studies show little vascular remodeling.\(^{78,80}\) A blunted pulmonary vascular pressor response to acute and sustained hypoxia is retained by Tibetans at sea level.\(^{81}\)

**Recent Genomic Studies in Humans**

A number of attempts have been made to understand adaptation to high-altitude life based on differences in candidate pathways, such as the ability of Tibetans to preserve NO production at altitude\(^{52}\) and candidate genes.\(^{66,83}\) This approach is selective and the data come from small subject numbers. The advent of high-throughput genome sequencing has enabled a less-biased strategy for investigating gene associations. The priority to date has been to compare the genetic architecture of people living at high altitude with that of lowlanders or recent migrants (genome-wide selection scans) rather than to compare well-phenotyped populations with and without pulmonary vascular disease at altitude (genome-wide association studies).

Seven genome-wide selection scans of Tibetan DNA have been reported. All have shown evidence of natural selection for noncoding variants in and around 2 HIF pathway genes, \(E\alpha P\alpha S1\) (HIF-2\(\alpha\)) and \(E\gamma N1\) (HIF prolyl 4-hydroxylase 2).\(^{84-90}\) Key to the interpretation of genetic data is robust phenotyping. Tibetans average \(\geq 1\) g/dL and as much as 3.5 g/dL (ie, \(\approx 10\%\) to \(20\%\) lower hemoglobin concentration compared with acclimatized lowlanders. At first sight, a paradox, a lower red cell mass by reducing blood viscosity may be an important factor enabling the cardiopulmonary circulation to adapt to high-altitude life. Significantly, the polymorphisms in \(E\alpha P\alpha S1\) and \(E\gamma N1\) in Tibetans correlate with hemoglobin concentration.\(^{84,86-88,90}\) A high-frequency missense mutation has recently been identified in \(E\gamma N1\) that encodes a variant prolyl 4-hydroxylase 2 with increased hydroxylase activity under hypoxic conditions that would contribute to this adaptive response.\(^{91}\)

A genome study in Andeans has found evidence of positive selection for \(E\gamma N1\) but not \(E\alpha P\alpha S1.\(^{92}\) Neither were candidates in reported studies in Ethiopian highlanders.\(^{93-95}\) Moreover, Andeans exhibit a robust erythropoietic response to altitude and polymorphisms identified in \(E\gamma N1\) in Andeans, albeit different from those in Tibetans, did not associate with hemoglobin level. Analysis of other quantitative traits, such as resting ventilation, hypoxic ventilator response, and oxygen saturation, also show differences between Tibetans and other Asian and European populations studied at the same altitude.\(^{96}\) It is likely that the Andean and Tibetan populations have developed different genetic adaptations to high-altitude hypoxia, although pathways may converge. In the case of Tibetans, one source of adaptation is likely to be attributed to the introduction of genetic variants from archaic Denisovian-like individuals into the ancestral Tibetan gene pool.\(^{97}\)

Aside from HIF, genes encoding downstream components of the HIF pathway remain a priori candidates for natural selection to hypoxia. A recent study of single nucleotide polymorphisms in 5 Ethiopian populations at altitude suggest positive selection for \(B\alpha L\beta H\epsilon 41\), a gene transcriptionally regulated by HIF-1\(\alpha\) and with a major regulatory role in the same hypoxia-sensing pathway described in Tibetans, indicative of convergent evolution.\(^{98}\) Other pathways may emerge from unbiased genome-wide studies in larger population cohorts. Evidence for adaptation outside the HIF family comes from a study of Eurasians exposed to mild-to-moderate hypoxia, where the strongest adaptive signal came from the \(\mu\)-opioid receptor-encoding gene (OPRM1, 2.54_10\(^{-9}\)).\(^{98}\)

Whole-genome sequencing of Andean highlanders, 10 with and 10 without CMS, followed by expression studies in fibroblasts identified 2 genes, \(S\alpha N\alpha P1\) and \(A\gamma N\alpha P32D\), that exhibit a higher transcription response to hypoxia in CMS subjects.\(^{99}\) Downregulation of the orthologs of these genes in flies enhanced their survival rates in a hypoxic environment. \(S\alpha N\alpha P1\) is known to regulate erythropoiesis and \(S\alpha N\alpha P1/-\) mice die early because of anemia, lending biological plausibility to this gene as a candidate for a role in CMS. There is widely believed to be a genetic predisposition to HAPE, but to date only candidate genes have been examined with no consensus observations.\(^{100}\)

**Conclusions**

Humans can live a normal life at high altitudes given sufficient time to acclimatize. People who exhibit a marked pulmonary vascular or erythropoietic response to hypoxia identify themselves as at risk of heart failure. Studies in Tibetan people adapted genetically to high altitude highlight the importance of the HIF pathway in determining the magnitude of response, but other pathways may emerge from studies of Tibetan cohorts better phenotyped for pulmonary hemodynamics, as well as studies of other ethnic groups. Considerable progress has been made in understanding the pathology of HAPH, but few drugs studied in animal models have been formally trialed in humans.

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None.


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Martin R. Wilkins, Hossein-Ardeschir Ghofrani, Norbert Weissmann, Almaz Aldashev and Lan Zhao

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