

The pulmonary vasculature – lessons from Tibetans and from rare diseases of oxygen sensing

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New findings

What is the topic of this review?

This review is principally concerned with results from studies of the pulmonary vasculature in humans, particularly in relation to hypoxia and rare diseases affecting oxygen sensing.

What advances does it highlight?

This review highlights the degree to which the hypoxia-inducible factor (HIF) transcription system influences human pulmonary vascular responses to hypoxia. Upregulation of the HIF pathway augments hypoxic pulmonary vasoconstriction (HPV), and alterations to the pathway found in Tibetans are associated with suppression of the progressive increase in pulmonary artery pressure with sustained hypoxia. It also highlights the potential importance of iron, which modulates the HIF pathway, in modifying the pulmonary vascular response to hypoxia.

Abstract

The human pulmonary circulation loses its natural distensibility under sustained hypoxia, leading to pulmonary arterial hypertension and a much higher workload for the right ventricle. The hypoxia-inducible factor (HIF) pathway is implicated in this pulmonary vascular response to continued hypoxia by animal studies, and additionally by rare human diseases where the pathway is upregulated. However, there are no known human genetic diseases downregulating HIF. Tibetans, though, demonstrate blunted pulmonary vascular responses to sustained hypoxia. This seems to be accounted for by an altered HIF pathway as a consequence of natural selection over a period of many thousands of years lived at high altitude. In addition to genetic differences, iron is another important modulator of HIF pathway function. Experimental work in humans demonstrates that manipulation of iron stores can influence the behaviour of the pulmonary circulation during hypoxia, in ways analogous to that seen in Tibetans and patients with rare diseases affecting oxygen sensing. The importance of physiological differences in iron bioavailability in modulating hypoxic pulmonary vasoconstriction in health and disease is yet to be established.

Text

Introduction

At sea level, the pulmonary vascular bed in healthy humans is a low pressure, highly distensible system which allows cardiac output to rise several-fold during exercise with only a small increase in pulmonary artery systolic pressure (PASP). As first reported nearly seventy years ago (von Euler & Liljestrand, 1946), the pulmonary vasculature constricts in response to hypoxia. Provided the hypoxic exposure is relatively brief, this aspect of hypoxic pulmonary vasoconstriction (HPV) is rapidly and completely reversible. However, if the hypoxic exposure is sustained, then there is a far greater rise in pulmonary artery pressure, a loss of distensibility of the pulmonary vascular bed, and very limited reversibility of response following removal of the hypoxic stimulus – at least in the short term. These effects are exceedingly well illustrated by data from serial pulmonary artery catheterisations in humans as part of the Operation Everest II Study (Groves *et al.*, 1987), which recorded pulmonary vascular responses to progressive hypobaric hypoxia over several weeks (Figure 1).

Two questions arise from the data in Figure 1. First, what is the time course over which the pulmonary vascular response to hypoxia changes from that of mild reversible vasoconstriction to a more powerful response which is not fully and rapidly reversible by the removal of the hypoxic stimulus? Secondly, what underlying mechanism causes the more powerful, sustained response to continued hypoxia?

Temporal characteristics of hypoxic pulmonary vasoconstriction

It is well recognised that some of the acclimatisation responses to hypoxia, whilst not immediate, occur relatively rapidly with significant changes observed over a period of just hours. To explore whether this was the case for the human pulmonary vascular response to hypoxia, healthy volunteers, in whom pulmonary artery catheters had been positioned, were exposed to 8 hours of normobaric hypoxia (Dorrington *et al.*, 1997). Isocapnic conditions were used to avoid confounding effects of a change in carbon dioxide on the pulmonary vasculature, secondary to changes in ventilation whilst hypoxic. The responses are shown in Figure 2. Of note is the modest initial rise in pulmonary vascular resistance by one hour; the progressive rise in resistance over the

next few hours; the failure of pulmonary vascular resistance to return immediately to normal levels after a return to euoxia; and the sensitisation of the acute response to a repeat hypoxic exposure. Thus the second phase of HPV, a significant progressive response to hypoxia, is observed only after a few hours of exposure (Talbot *et al.*, 2005).

Inserting pulmonary artery catheters into healthy humans for the purposes of physiological research is relatively invasive. Since Doppler echocardiography was first shown to be practical for non-invasive assessment of the pulmonary circulation (Yock & Popp, 1984), important advances have been made in the use of this technique which has been widely employed to address the physiology of the pulmonary circulation during hypoxia by ourselves and others (Peacock *et al.*, 1990; Talbot *et al.*, 2005; Smith *et al.*, 2008; Smith *et al.*, 2009; Sable *et al.*, 2011; Foster *et al.*, 2014). The remainder of the work in this review has used echocardiography to assess pulmonary artery pressure.

The hypoxia-inducible factor (HIF) pathway

The time course of pulmonary vasoconstriction seen in response to prolonged hypoxia, taken with the sensitisation effect on further acute hypoxic exposures, suggests that changes in gene expression may underlie the second phase of HPV. The HIF pathway, which regulates the expression of several hundred genes in response to changes in oxygen levels, including many important for angiogenesis, erythropoiesis and cellular metabolism (Semenza, 2011), is an obvious candidate mechanism. The HIF transcription factor protein complex is composed of two subunits designated HIF- α and HIF- β ; the HIF- α subunit is regulated by oxygen tension and the heterodimer is active as a transcription factor. HIF- α is hydroxylated by prolyl-hydroxylase domain enzymes (PHDs) which require dioxygen and 2-oxoglutarate as substrates. Once hydroxylated, HIF- α can be bound by another protein, the von Hippel–Lindau (VHL) protein, which targets HIF- α for proteasomal degradation. Since the PHDs require oxygen as a substrate, under hypoxic conditions this process is inhibited and HIF- α accumulates. The PHD enzymes also depend on a single ion of ferrous iron at their catalytic centres. Figure 3 gives a schematic representation of the regulation of HIF levels by oxygen availability.

In humans there are three paralogues of HIF- α with slightly different roles, HIF-1 α , HIF-2 α and HIF-3 α . The regulation of the two main paralogues (HIF-1 α and HIF-2 α) differs, and the balance between them may be important for controlling tissue-specific differences in responses to hypoxia (Semenza, 2011). The importance of the HIF pathway for the behaviour of the pulmonary vasculature during hypoxia is confirmed by the findings that heterozygous HIF-1 α deletion attenuates the development of hypoxic pulmonary hypertension in mice (Yu *et al.*, 1999), whereas mice hetero- or homozygous for a mutation stabilising HIF-2 α spontaneously develop pulmonary hypertension in a gene dose-dependent manner (Tan *et al.*, 2013). From an understanding of the molecular biology of the pathway, it might also be anticipated that naturally occurring mutations in pathway components such as VHL, the PHDs and HIF itself would exist with subtle effects on oxygen sensing. Given the range of altitudes over which human habitation is seen, and the profound sustained hypobaric hypoxia to which several populations must have adapted as a result, it would not be surprising if one or more of these putative genetic variants conferred some advantage for life in these environments. As iron is a necessary cofactor for the hydroxylation of HIF- α by the PHDs, it might also be the case that changes in iron bioavailability have an effect on oxygen sensing and signalling behaviours.

Chuvash Polycythaemia: the archetypal rare disease of oxygen sensing

Chuvash Polycythaemia (CP) is a rare autosomal recessive condition in which a hypomorphic form of VHL, which cannot properly bind HIF- α , is inherited. The α -subunit therefore escapes proteasomal degradation despite euoxia and a hypoxia signal is generated at normal oxygen tensions. Affected individuals show elevated resting PASP and an exaggerated pulmonary vasoconstrictive response to mild and moderate hypoxia (Smith *et al.*, 2006), as illustrated in Figure 4. Elevated resting ventilation and ventilatory responses to hypoxia, along with marked polycythaemia, are also features of CP. Genes under the transcriptional control of HIF, such as aldolase C, also appear to be upregulated compared to healthy controls over a range of oxygen tensions. In this way a subtle modification of a single HIF pathway component can be seen to retune oxygen sensing in a way which affects integrated cell and systems-level physiological responses to hypoxia. Mutations in other pathway components have also been identified with more

limited effects than those seen in CP patients (Formenti *et al.*, 2011); some appear significantly to affect only erythropoiesis (Lee & Percy, 2011).

Lessons from Tibetans

The available evidence supports the view that the Tibetan Plateau is the longest continuously-inhabited high-altitude region of the Earth (Aldenderfer, 2011; Qi *et al.*, 2013). Tibetans have inhabited this environment, with an elevation of around 4000 m and inspired partial pressure of oxygen of approximately 80 mmHg, for in excess of 25,000 years. A study comparing Tibetan residents at 3,200 – 3,500 m in Yunnan Province, China with Han Chinese as controls, identified eight single nucleotide polymorphisms (SNPs) close to one another and the gene *EPAS1* as having undergone selection within the Tibetan population (Beall *et al.*, 2010) (Figure 5). *EPAS1* encodes HIF-2 α , one of the three oxygen-regulated HIF- α paralogues in humans. Sex-adjusted haemoglobin concentrations were, on average, 0.8 g/dL lower for individuals homozygous for the major alleles compared with heterozygotes. What, then, apart from a difference in haemoglobin concentration, are the physiological consequences of selection pressure on this genomic region for Tibetans? To address this question, the effect of 8 hours of normobaric hypoxia on acute HPV was studied in Tibetans and Han Chinese controls living at sea-level in the UK (Petousi *et al.*, 2014).

Figure 6 shows the pulmonary vascular responses of both groups before and after an eight-hour long hypoxic exposure, during which end-tidal partial pressures of oxygen were held at 50 mmHg. Prior to this exposure, the responses of Tibetans and Han Chinese to an acute hypoxic challenge were quite similar. Following the long hypoxic exposure however, PASP was elevated in the Han Chinese in euoxia and rose much more vigorously than it had done beforehand when a second acute hypoxic challenge was given. The Tibetans, though, did not show this elevation in PASP whilst euoxic following the long hypoxic exposure; the acclimatisation effect was not evident. Moreover, the rise in PASP during the second acute hypoxic challenge, though still greater than at the outset of the experiment, was nothing like as profound as that seen in the Han group. This hyporesponsiveness of the pulmonary circulation mirrors the attenuated polycythaemic response seen in Tibetans living at high altitude. In fact, even at sea level the Tibetans had a haemoglobin concentration around 1 g/dL lower than

Han controls. Additionally, HIF target genes including aldolase C appeared to be under-expressed in peripheral blood lymphocytes (PBL) from the Tibetan group compared to Han Chinese over a range of levels of hypoxia. HIF-2 α mRNA expression was correspondingly lower in PBL from the Tibetans than Han Chinese; mRNA levels of *EGLN1*, the gene which encodes PHD2, did not differ.

Iron and the pulmonary vasculature

Iron has long been known to be important for oxygen sensing. In fact, work implicating iron in the regulation of expression of the erythropoietin gene led to the expectation that the oxygen sensor responsible, when discovered, would be a heme protein (Goldberg *et al.*, 1988). When HIF was characterised as the relevant transcription factor, it was shown that iron-chelation using desferrioxamine could induce HIF-1 activity and erythropoietin gene expression *in vitro* with a similar time course to that observed with hypoxia (Wang & Semenza, 1993). Lowering iron availability was therefore shown to have a hypoxia-mimetic effect.

To answer the question of whether significant experimentally-induced changes in iron bioavailability have an effect on HPV in humans, a series of experiments were conducted using iron chelation and iron loading. In the first instance, it was possible to show that, in humans breathing air, infusion of desferrioxamine resulted in a significant increase in erythropoietin expression (Ren *et al.*, 2000) as well as a rise in PASP (Balanos *et al.*, 2002). Interestingly, no significant changes in ventilation were seen; it may be that the influence of the HIF pathway on these two different aspects of hypoxia biology differs in a fundamental way. In later experiments, the contrasting effects of iron depletion and supplementation on HPV in both normobaric and hypobaric hypoxia were shown. In a cross-over study (Smith *et al.*, 2008), loading healthy individuals with intravenous iron prior to a long hypoxic exposure resulted in abrogation of the elevation in PASP when participants returned to air-breathing following eight hours of hypoxia. Additionally, the rise in PASP during a subsequent acute hypoxic challenge was much lower when iron loading was given compared with placebo. Iron thus interfered with the acclimatisation of the pulmonary vasculature to sustained hypoxia, making Caucasians behave effectively like Tibetans (Figure 7). Similarly, at high altitude, infusion of iron produced a fall in PASP within hours even though individuals had been exposed to

hypobaric hypoxia for several days (Smith *et al.*, 2009). Conversely, in the same study, progressive iron deficiency induced by repeated venesection in individuals with chronic mountain sickness resulted in a significant rise in PASP.

Whether naturally occurring iron deficiency is associated with detectable differences in HPV in healthy humans is the subject of work that is currently ongoing. Considerable interest exists in clarifying the role of iron deficiency in the aetiology of primary and secondary pulmonary hypertension (Rhodes *et al.*, 2011; Ruiter *et al.*, 2011; Soon *et al.*, 2011; Ruiter *et al.*, 2014; van Empel *et al.*, 2014), particularly whether intravenous iron might be a novel therapeutic approach for patients with these difficult-to-treat conditions (Howard *et al.*, 2013). Given that even in the setting of established pulmonary arterial hypertension it seems probable that there remains a significant degree of reversibility (Rowan & McLoughlin, 2014), this work is particularly exciting.

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Additional information

Competing interests

PAR has received funding from Vifor Phama, the manufactures of Ferinject®, an intravenous iron preparation, for research studies not presented in this review.

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Figures

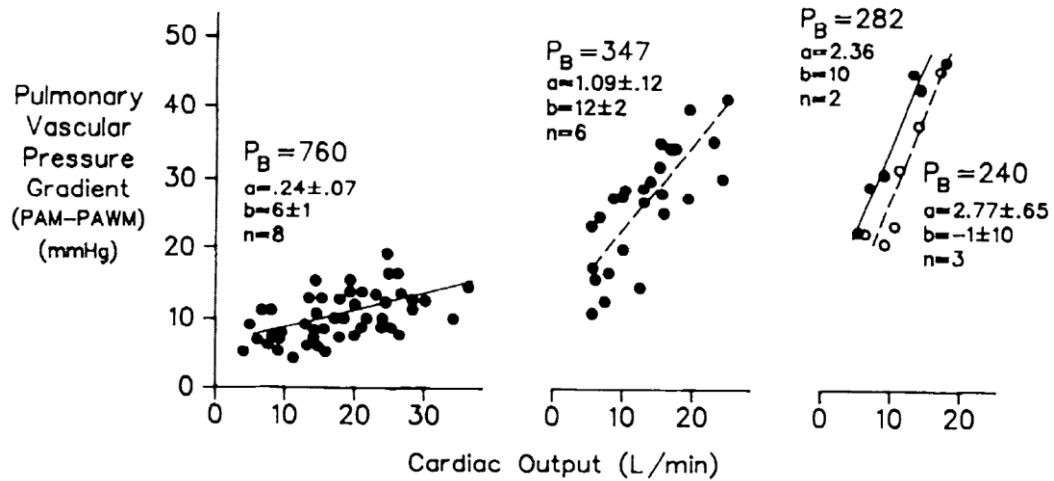


Figure 1. Relationship between mean pulmonary pressure gradient (pulmonary arterial mean pressure (PAM) minus pulmonary arterial wedge mean pressure (PAWM)) and cardiac output over a range of barometric pressures (P_B) (Torr). The average regression lines ($y = ax + b$) for the group (n individuals) are given for each P_B . Reproduced with permission from (Groves *et al.*, 1987).

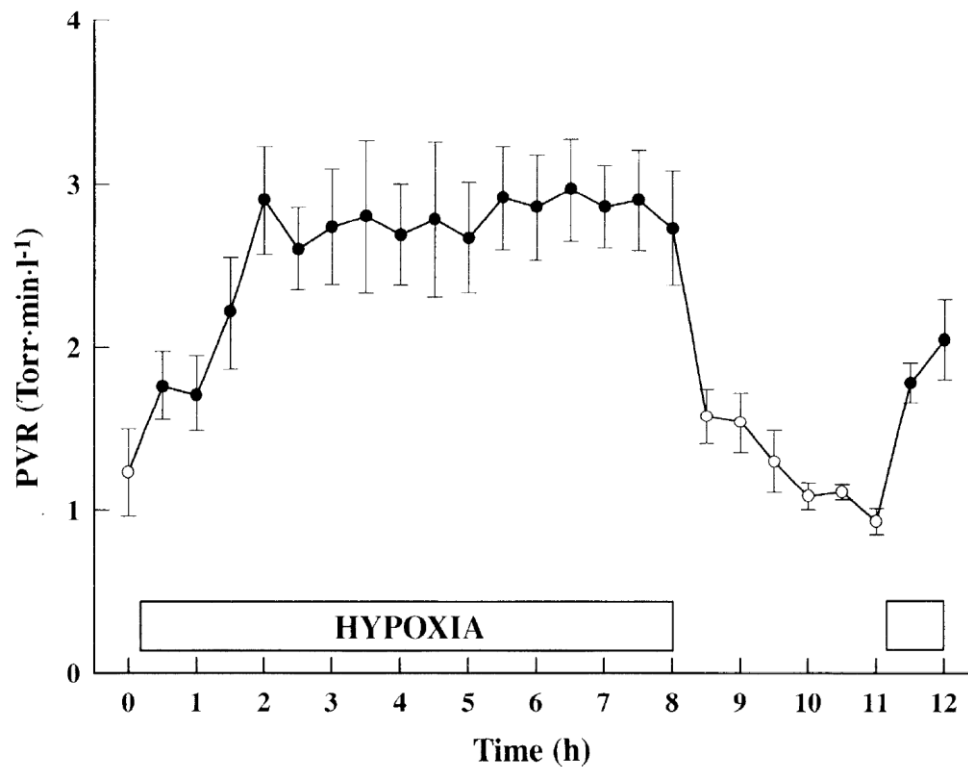


Figure 2. Pulmonary vascular resistance (PVR) measured in six subjects during a twelve hour period in a purpose-built chamber, during which time they were exposed to 8 hours of isocapnic hypoxia (end-tidal partial pressure of oxygen 50 Torr), 3 hours of isocapnic euoxia, and finally one further hour of isocapnic hypoxia. Values are means \pm SE. Reproduced with permission from (Dorrington *et al.*, 1997).

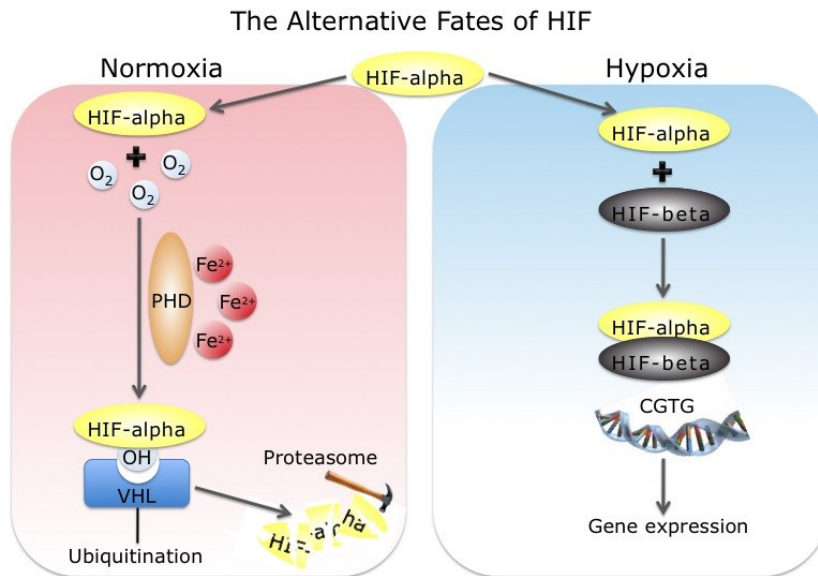


Figure 3. Regulation of HIF- α levels by oxygen availability. In euoxia, HIF- α is rapidly hydroxylated and broken down. Under hypoxic conditions, HIF- α accumulates and is able to translocate to the nucleus, bind HIF- β and function as a transcription factor. Courtesy of Dr Federico Formenti, University of Oxford.

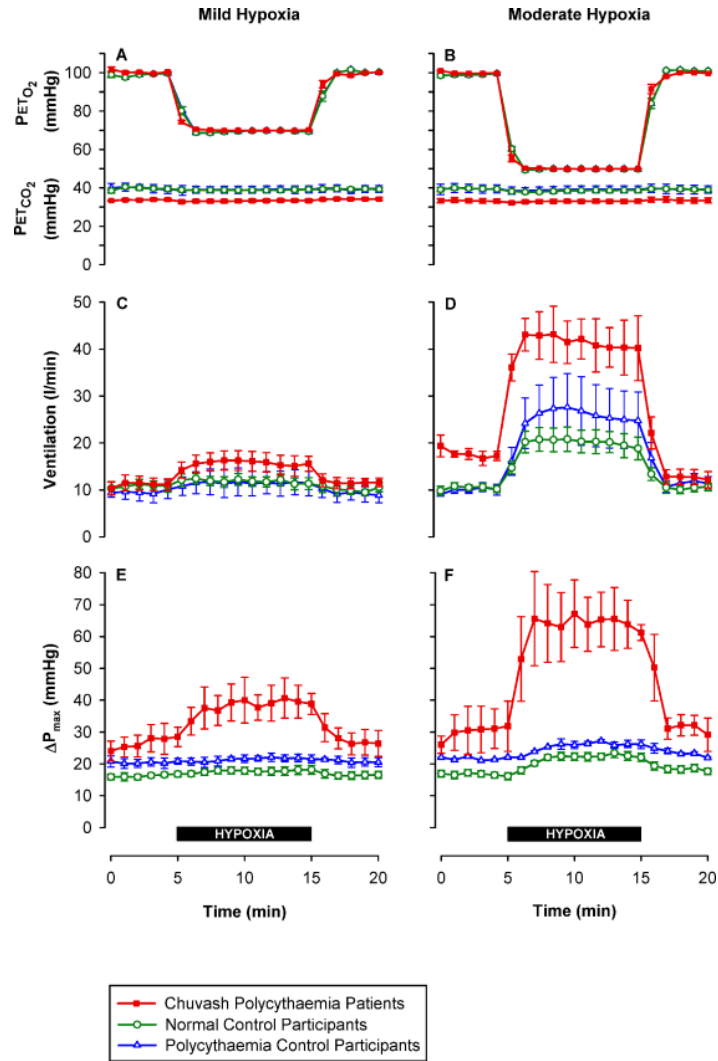


Figure 4. Ventilation (C and D) and pulmonary vascular responses (E and F) during mild and moderate hypoxia (A and B), in patients with Chuvash Polycythaemia and normal controls. A third group of patients with polycythaemia rubra vera served as additional controls given the elevated haematocrit in the CP group. Mild hypoxia provoked an increase in ventilation of 4.4 L/min in the CP patients versus 1.6 L/min in normal controls ($P < 0.05$), while moderate hypoxia induced increases of 24.5 L/min versus 10.0 L/min in these two groups, respectively ($P < 0.05$). ΔP_{max} , an index of PASP, increased by 11.5 mmHg in the CP group during mild hypoxia, compared with only 1.1 mmHg in the control group ($P < 0.05$). Moderate hypoxia stimulated a rise in ΔP_{max} of 35.3 mmHg compared with 6.1 mmHg in these two groups, respectively ($P < 0.001$). Values are mean \pm SE. Reproduced from (Smith *et al.*, 2006).

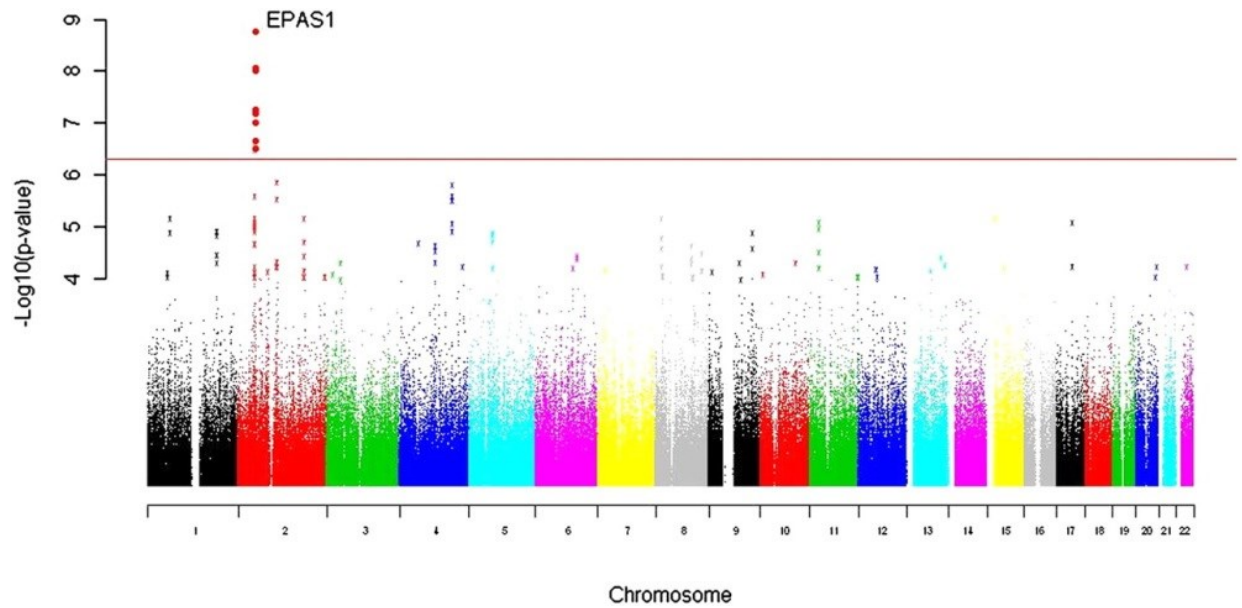


Figure 5. Genome-wide allelic differentiation scan comparing Tibetans with Han Chinese. The horizontal axis indicates genomic position and the different colours, chromosomes. The vertical axis indicates negative log of SNP-by-SNP P values generated from a Tibetan versus HapMap Han Chinese comparison. The red line indicates the threshold for genome-wide significance ($P = 5 \times 10^{-7}$). Values are shown after correcting for background population stratification using genomic control. Reproduced from (Beall *et al.*, 2010).

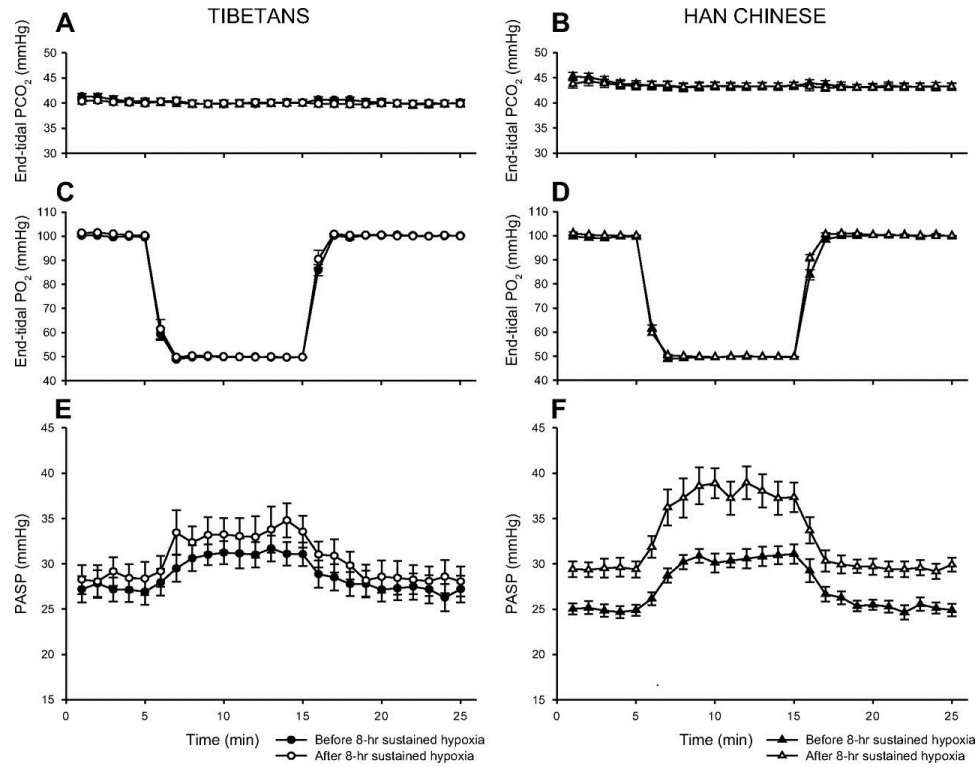


Figure 6. Pulmonary vascular responses to acute isocapnic hypoxia before and after an eight hour sustained isocapnic hypoxic exposure. (A and B) End-tidal partial pressure of carbon dioxide over time. (C and D) End-tidal partial pressure of oxygen over time. (E and F) PASP values over time. All data are for 10 Tibetan and 10 Han Chinese volunteers, respectively. Values are means; error bars represent SE. Reproduced with permission from (Petousi *et al.*, 2014).

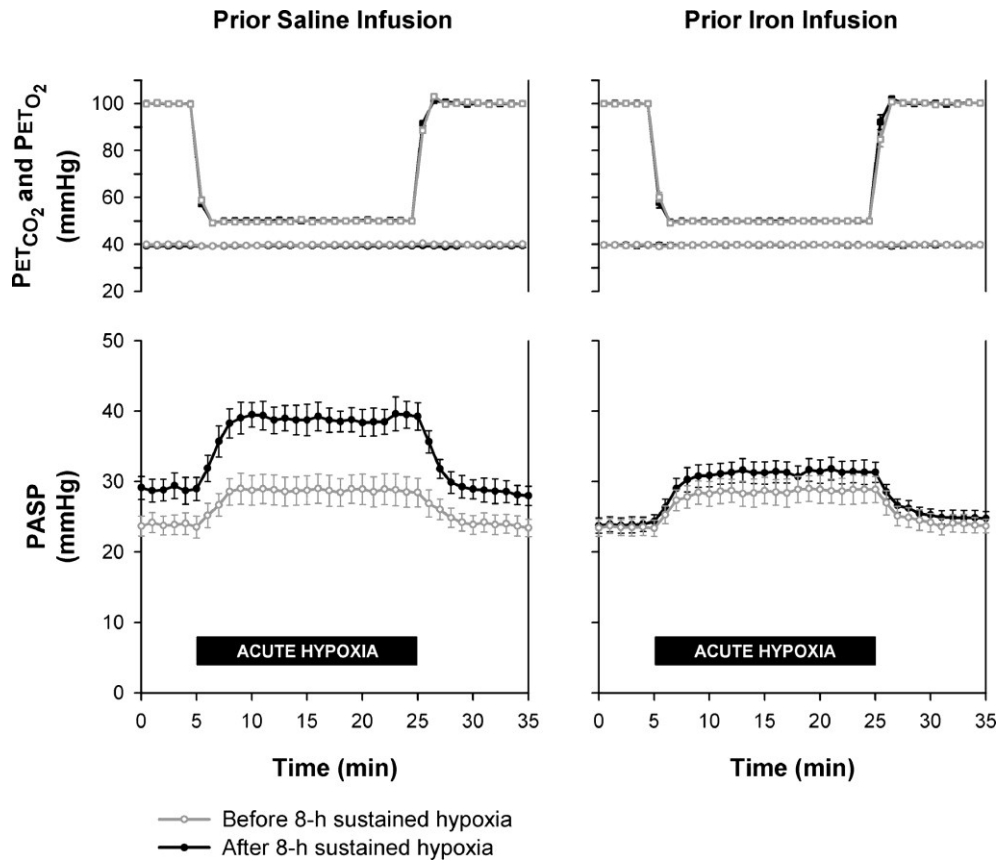


Figure 7. Effects of 8 hours of sustained hypoxia on the pulmonary vasculature with and without prior infusion of iron. Upper panels show end-tidal partial pressures of oxygen and carbon dioxide during two acute hypoxic challenges. Lower panels show corresponding PASP measurements. Values are means; error bars SE. The control measurements demonstrate the normal pulmonary vascular response to sustained hypoxia, consisting of an increase in PASP and a sensitized PASP response to subsequent acute hypoxia. Intravenous infusion of iron prior to the 8 hour hypoxic exposure prevented the increase in baseline PASP ($P < 0.001$) and markedly attenuated the degree to which the vasculature was seen to be sensitised on repeat acute hypoxic exposure ($P = 0.002$). Reproduced with permission from (Smith *et al.*, 2008).