REVIEW

Heights and haematology: the story of haemoglobin at altitude

Jeremy S Windsor, George W Rodway

Postgrad Med J 2007;**83**:148–151. doi: 10.1136/pgmj.2006.049734

In order to compensate for the low partial pressure of oxygen at altitude, the human body undergoes a number of physiological changes. A vital component in this process is the increase in the concentration of circulating haemoglobin. The role of HIF-1α, erythropoietin and red blood cells in this acclimatisation process is described, together with the fall in plasma volume that increases the concentration of haemoglobin in the early stages of hypoxic exposure.

See end of article for authors' affiliations

Correspondence to: Jeremy S Windsor, Centre for Aviation, Space and Extreme Environment Medicine (CASE), University College London, Archway Campus, Whittington Hospital, Archway, London N19 5NF, UK; jswindsor@doctors.org.uk

Received 18 May 2006
Accepted
13 November 2006

Although estimates vary, it is thought that approximately 140 million people live above 2500 m, with the majority being found in Central Asia, East Africa, Central and South America (table 1).[1]

The greatest challenge facing humans at altitude is the reduction in the partial pressure of oxygen that results from a fall in barometric pressure. When faced with this hypoxic challenge, the body responds in a number of different ways depending upon the rate and severity with which the stimulus is imposed. The acute hypoxia suffered by aviators in an unpressurised aircraft generates a very different set of physiological responses than the more chronic form experienced by mountaineers, who typically take several weeks to reach similar heights. It is striking to note that someone who ascends rapidly from sea level to the summit of Mt Everest (8850 m) will lose consciousness within seconds, while those who have spent several weeks ascending can often function relatively well. The physiological changes which allow this to occur are grouped together under the term ''acclimatisation'', while change that occurs over many generations in high altitude populations is known as ''adaptation''. In order to cope with hypoxia, the body attempts to maximise the delivery of oxygen to the tissues. Within minutes of arriving at altitude this is manifest by an increase in cardiac output and minute ventilation.[2] Over time, additional improvements occur in both the circulation and tissues that enhance the acclimatisation process further. This review will focus upon perhaps the most widely known change seen on ascent to altitude, that is the increase in the concentration of haemoglobin.

Early historical developments

In 1674 Anthony Von Leeuwenhoeck announced to members of the Royal Society, ''I have observed, taking some blood out of my hand, that it consists of small round globules driven through a crystalline humidity of water''.[3]

This, the first recorded description of a red blood cell (erythrocyte), would provide the starting point for a clear description of the constituents of the cell and finally, an understanding of its origins and function. At the University of Bologna in 1747, Vincenzo Menghini demonstrated the presence of iron in red cells by burning blood and showing that its ashes were attracted to a magnet.[3] Later, Jons Jacob Berzelius distinguished between the protein ''globulin'' and the pigmented ''haem'' compound contained in the cell, before correctly identifying that the latter component carried the iron moiety.[4] Subsequent experiments conducted by Johannes Mulder determined the composition of the ''haem'' component and demonstrated that the pigmented structure was responsible for carrying oxygen.[5] By the middle of the nineteenth century, Felix Hoppe Seyler was able to crystallise the molecule and finally named it ''haemoglobin''. Seyler would later go on to describe the formation of ''oxyhaemoglobin'' following the reaction of haemoglobin with oxygen.[6] In 1865 Seyler presented his results to his colleague Paul Bert (fig 1). By exposing animals to a range of different barometric pressures, Bert was able to describe for the first time a rudimentary oxyhaemoglobin dissociation curve.[7]

Bert is considered by many to be the father of high altitude physiology. With the publication of *La Presson Bariometrique* in 1878, Bert was the first to make the connection between the problems humans faced at high altitude and the fall in barometric pressure.[8] This work had been prompted in large part by fellow Frenchman and physician Denis Jourdanet.[9] Jourdanet had spent almost 20 years practising medicine at high altitudes in Mexico and had focused much of his attention on the effects of high altitude on humans. With Jourdanet's subsequent encouragement and financial support, Bert was able to build one of the earliest pressure chambers in his laboratory and was able to demonstrate that by breathing supplemental oxygen in hypoxic conditions, the symptoms of acute mountain sickness could be treated.

During his time in Mexico, Jourdanet observed a consistent increase in the viscosity of human blood at high altitude. This would lead Bert to correctly hypothesise that such a change was due to an increase in the concentration of red cells in the circulation. However, it would take another

Abbreviations: CMS, chronic mountain sickness; EPO, erythropoietin; HIF-1α, hypoxia inducible factor-1α; HVR, hypoxic ventilatory response; VHL protein, Von Hippel Lindau protein

Table 1 The five countries with the largest populations living above 2500 m[1]

Country	Estimated population living above 2500 m (millions)	Estimated percentage of the total population (%)
India	26.82	3
China	22.09	2
Mexico	14.05	15
Pakistan	14.05	10
Ethiopia	13.76	25

Frenchman, Francois-Gilbert Viault to confirm this, when in 1890 he found that after 23 days at 4392 m, his own red cell count had risen from 5 to 8 million per cubic millimetre.[10] In later studies on lowland Europeans and Peruvian high altitude residents, Viault was able to confirm what is now commonly known – haemoglobin concentrations rise on ascent to altitude.

Twentieth century and beyond

The response of lowlanders to hypoxia

In 1906 Carnot and Deflandre, two scientists at the University of Paris, took the understanding of haemoglobin one step further. By injecting the serum of bleeding rabbits into otherwise healthy animals, they were able to demonstrate an increase in the red cell concentrations of the transfused animals. This, the authors felt, had shown ''the presence of a substance haemopoietin [erythropoietin] capable of activating haemopoiesis and of rapidly provoking in normal animals, a high constant hyperglobulism''.[11]

Although it would take almost 50 years to demonstrate the effect of erythropoietin (EPO) on erythroid cell lines in humans, the structure and origins of the molecule would soon follow. However, it was not until the discovery of hypoxia inducible factor-1α (HIF-1α), largely as a result of Greg Semenza's work in the 1990s, that the agent responsible for EPO release was discovered.[12 13] Under normoxic conditions, HIF-1α has one of the shortest half lives of any normal protein, binding to the Von Hippel Lindau (VHL) protein inside healthy cells before rapidly degrading.[14] However, under hypoxic conditions HIF-1α resists VHL and instead binds to HIF-1β to form HIF.[15] This molecule subsequently binds to the EPO gene on chromosome 7 and stimulates red cell production. In Chuvash polycythaemia, a recessive condition common in parts of the former Soviet Union, abnormalities in the VHL protein prevent HIF-1α from degrading under normoxic conditions.[16] The consequences for those with this condition are the same as those seen in individuals ascending to altitude – an increase in HIF, EPO and red cell production.

As a protein that is being constantly being produced and degraded in cells, HIF-1α responds to hypoxia rapidly. Within an hour of exposure to hypoxia, HIF-1α concentrations peak and trigger an almost immediate rise in the concentration of circulating EPO.[17] Increases in EPO are strongly related to the altitude reached, with a rise of 30% at 1900 m compared to 300% at 4500 m.[18] Interestingly, absolute values can vary by as much as +400% to −40% among individuals exposed to 3000 m over a period of 24 h.[19] The reasons for this variation are unclear, although it is thought that subtle polymorphisms in the EPO gene may make a significant contribution.[20] During a prolonged stay at altitude, EPO levels continue to rise for up to 3 days before eventually returning to normal after 3 weeks.[21] Recently, it has been suggested that this fall may be relative, with production of EPO remaining high throughout and the consumption of EPO gradually increasing over the first month of exposure. This would result in an apparent fall in EPO concentration, despite EPO production remaining high.[22] Once bound to the erythroid cells in the bone marrow, EPO triggers an increase in iron turnover and a doubling of nucleated red cells within 7 days of exposure to moderate altitude.[23] In a long term study conducted over a year, the increase in red cell production has been shown to continue for up to 8 months and result in an increase in red cell mass of 50%.[24]

Figure 1 Paul Bert, considered by many to be the father of high altitude physiology.

Despite such an enormous change, the increase in haemoglobin concentration appears to be even more dramatic during the first few weeks spent at altitude. The reason for this was first identified in 1952 by Lawrence *et al* who measured changes in the circulation with autologous red cells labelled with radioactive phosphorous and found that plasma volume fell shortly after ascending to altitude.[25] This was confirmed almost a decade later by Pugh, the physiologist assigned to the first successful expedition to Mt Everest in 1953 and the scientific leader of the landmark ''Silver Hut'' scientific expedition to the Khumbu region of Nepal in 1960–1[26] (fig 2).

Figure 2 The ''Silver Hut'' expedition, a landmark in high altitude research. Photograph courtesy of JB West.

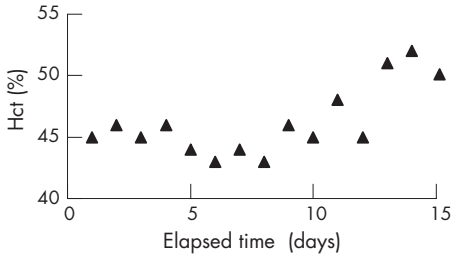

Figure 3 The mean values of haematocrit (Hct) seen in four mountaineers ascending from 1530 m to advanced base camp (5700 m) on Cho Oyu (8201 m).

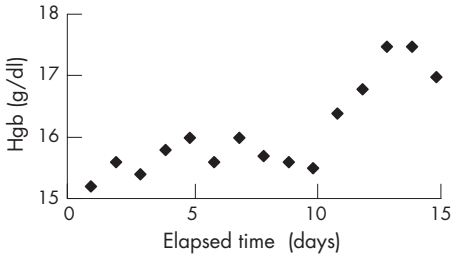

Figure 4 The mean values of haemoglobin (Hgb) concentration seen in four mountaineers ascending from 1530 m to advanced base camp (5700 m) on Cho Oyu (8201 m).

Following 18 weeks above 4000 m, Pugh identified a 21% reduction in the plasma volume of four healthy expedition members. However over the following 3 months this deficit narrowed and resulted in just a 10% reduction by the end of the expedition.[26] This transient fall in plasma volume has the potential to provide mountaineers with an important boost during the first few weeks at altitude. Although volumes of haemoglobin are only just starting to rise, a sudden reduction in plasma volume can rapidly increase the concentration of haemoglobin and therefore enhance the carriage of oxygen for any given volume of blood.

Previously unpublished data from the 2005 Xtreme Everest Expedition to Cho Oyu (8201 m) demonstrate that large increases in haematocrit and haemoglobin concentration occurred during the 15 day journey from Kathmandu (~1530 m) to advanced base camp (~5700 m) (figs 3 and 4).

A rapid increase in the concentration of haemoglobin provides mountaineers with a means to compensate for the dramatic fall in arterial oxygen saturation seen at altitude (table 2).

Although a small increase in haemoglobin would normally be expected over the first few weeks at altitude, the increase in concentration seen here (approximately 2 g/dl) is largely due to a redistribution of total body water, with fluid being shifted from the circulation and deposited into the interstitial space. Unfortunately, the factors responsible for this shift are unclear and despite a number of different studies that have examined the behaviour of the sympathetic nervous system and a range of different hormones at altitude, we are still no closer to explaining this phenomenon.

The response of high altitude residents to hypoxia

As Viault demonstrated more than a century ago, sea level and high altitude residents both experience an increase in haemoglobin on ascending to higher altitudes. Although the final concentration can vary, the increase is largely dependent upon the altitude reached and the individual's arterial oxygen saturation.[27] Underlying this similarity is the knowledge that the structure and function of haemoglobin molecules vary little between different ethnic groups. Although the incidence of haemoglobinopathies (such as sickle cell disease and β-thalassaemia) may vary between populations, the behaviour of HIF, EPO and red cells when exposed to hypoxia is broadly similar.[1] Despite this, considerable variation exists in the final concentration of haemoglobin between different ethnic groups resident at moderate altitude. Over the last two decades Cynthia Beall and her colleagues have conducted a number of meticulous studies comparing the haemoglobin concentrations of residents in Bolivia, Tibet and Ethiopia living at altitudes of 3500–4000 m[29–32] (table 3).

In a comparison between Bolivian and Tibetan residents, Beale and her colleagues were able to control for a range of potential conflicting factors including concurrent medical illnesses, dietary deficiencies, occupation, cigarette smoking and exposure to fossil fuel smoke. This subsequently allowed the authors to demonstrate that genetic factors accounted for more than 85% of the difference between the two groups and led them to conclude that the difference was due to Tibetans undergoing a much longer period of adaptation. Although high altitude residents have been present in the Altiplano regions of the Andes for approximately 9000–12 000 years, the Himalayan plateau has been populated by humans for more than 50 000 years and it is this difference that has provided Tibetans with a longer period to adapt to hypoxia and subsequently develop a lower concentration of haemoglobin.

A normal haemoglobin concentration is vital for longevity and is well demonstrated by the huge variance in life expectancy (42 *v* 70 years) between those with Chuvash polycythaemia and matched controls.[16] This enormous difference is largely due to the increase in viscosity caused by the high concentration of red cells in the circulation that results in an increased incidence of heart failure and thrombo-embolic disease. Over the course of 50 000 years, Tibetan residents have undergone considerable natural selection that has discouraged the survival and reproductive success of those with high haemoglobin concentrations. By comparison, the Andean natives, whose ancestors moved to high altitude relatively recently, have not yet achieved an equivalent level of adaptation. This is compounded by the widespread colonisation of Andean communities which has led to out-breeding with low altitude residents.

In order to maintain low levels of haemoglobin and still deliver adequate amounts of oxygen to the tissues, Tibetan residents have made a unique adaptation. Following exposure to low partial pressures of oxygen, humans respond by increasing the rate and depth of their breathing in order to ensure adequate oxygenation. Although this hypoxic ventilatory response (HVR) varies considerably between humans, a pattern has emerged. In a comparison between Tibetan and Andean high altitude residents, Tibetans have been found to have a higher HVR and as a consequence an increase in resting minute ventilation.[32] This would suggest that the blunted HVR

Table 2 Content of oxygen in the blood at 1530 m and at Cho Oyu advanced base camp (5700 m)

	Day 1: 1530 m	Day 14: 5700 m
Arterial oxygen saturation (SaO_2)	95	82
Haemoglobin concentration (g/dl)	15.2	17.5
Oxygen delivered by haemoglobin to the tissues (ml/100 ml blood)	19.3	19.2

Despite a fall in arterial oxygen saturation, the content of oxygen in the blood remains unchanged at Cho Oyu advanced base camp (5700 m) due to a significant increase in haemoglobin concentration.

Table 3 Haemoglobin concentrations in residents living at 3500–4000 m and at sea level

	Haemoglobin in males (g/dl)	Haemoglobin in females (g/dl)
Sea level	15.3	13.4
Bolivia	17.9	16.8
Tibet	16.7	15.0
Ethiopia	15.9	15.0

Haemoglobin concentrations collected from studies of high altitude residents living at altitudes of 3500–4000 m compared to sea level residents from the USA.[28 30]

of Andean residents might limit the delivery of oxygen to the tissues and therefore promote an increase in red cell production with potentially detrimental effects. A low HVR also contributes to the development of chronic mountain sickness (CMS), a condition found in some high altitude residents which is characterised by abnormally high concentrations of haemoglobin. The resulting clinical problems faced by those with CMS are similar to those seen in Chuvash polycythaemia.[33] Interestingly, current evidence seems to infer that CMS is much more common in the Andean population, suggesting that without sufficient ''time'' for adaptation, populations at altitude can face considerable difficulties.[34]

Unlike the genetic changes made by Tibetan high altitude residents, little is known about how Ethiopians have adapted to their environment. However, unlike Tibetans, Ethiopians have consistently high arterial oxygen saturations despite the hypoxic environment, suggesting that natural selection may have led to improved oxygen delivery to the circulation and an increase in oxygen affinity of red blood cells.[30]

CONCLUSION

Since Von Leeuwenhoeck's discovery of ''small round globules'' almost 400 years ago, scientists have been able to identify, in detail, the structure and function of haemoglobin. More recently, the discovery of HIF-1α has identified the trigger for increases in EPO and red cell production under hypoxic conditions. These developments will continue to provide the basis for future research that explores the process of acclimatisation at both the cellular and molecular level. This will focus not only on red blood cells but also on the tissues they supply. Fascinating research opportunities surely beckon!

Authors' affiliations

Jeremy S Windsor, Centre for Aviation, Space and Extreme Environment Medicine (CASE), University College London, London, UK
George W Rodway, Center for Sleep and Respiratory Neurobiology, University of Pennsylvania Schools of Medicine and Nursing, Philadelphia, USA

Competing interests: None.

REFERENCES

1 **Niermeyer S**, Zamudio S, Moore LG. The people. In: Hornbein TF, Schoene RB, eds. *High altitude – an exploration of human adaptation*. New York: Marcel Dekker, 2001:43–100.
2 **Ward MP**, Milledge JS, West JB. *High altitude medicine and physiology*. London: Arnold, 2000:44–9.
3 **Houston C**. *Going higher*. Seattle: The Mountaineers, 1998:57–8.
4 **Berzelius JJ**. *Föreläsninger över djurkemien*. Stockholm: Marquad, 1806–08, vol 1–2.
5 **Severinghaus JW**, Astrup P, Murray JF. Blood gas analysis and critical care medicine. *Am J Respir Crit Care Med* 1998;**157**:114–22.
6 **Hoppe-Seyler F**. ?ber das verhalten des blutfarbstoffes im spectrum des sonnenlichtes. *Arch Pathol Anat Physiol* 1862;**23**:446.
7 **West JB**. *High life: a history of high altitude physiology and medicine*. New York: Oxford University Press, 1998:40–73.
8 **Bert P**. *La pression barométrique: recherches de physiologie expérimentale*. Paris: Masson, 1878.
9 **Bert P**. *Barometric pressure: researches in experimental physiology*. Columbus, OH: College Book Company, 1943.
10 **Viault F**. On the large increase in the number of red cells in the blood of the inhabitants of the high plateaus of South America. In: West JB, ed. *High altitude physiology*. Stroudsburg, PA: Hutchinson Ross, 1981:333–4.
11 **Carnot P**, Deflandre C. Sur l'activite haemopoietique du serum. *Compt Rend Acad D Sc* 1906;**143**:432–5.
12 **Semenza GL**. Regulation of erythropoietin production. New insights into molecular mechanisms of oxygen homeostasis. *Hematol Oncol Clin North Am* 1994;**8**:863–84.
13 **Semenza GL**, Roth PH, Fang HM, *et al*. Transcription regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *J Biol Chem* 1994;**269**:23757–63.
14 **Wang GL**, Jiang BH, Rue EA, *et al*. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular oxygen tension. *Proc Natl Acad Sci U S A* 1995;**92**:5510–14.
15 **Wang GL**, Semenza GL. Molecular basis of hypoxia-induced erythropoietin expression. *Curr Opin Haematol* 1996;**3**:156–62.
16 **Gordeuk VR**, Sergueeva AI, Masnikova GY, *et al*. Congenital disorder of oxygen sensing: association of the homozygous Chuvash polycythaemia VHL mutation with thrombosis and vascular abnormalities but not tumours. *Blood* 2004;**103**(10):3924–32.
17 **Schmidt W**. Effects of intermittent exposure to high altitude on blood volume and erythropoietic activity. *High Alt Med Biol* 2002;**3**(2):167–76.
18 **Mottram DR**. *Drugs in sport*, 4th edn. London: Routledge, 2005:229.
19 **Ri-Li G**, Witkowski S, Zhang Y, *et al*. Determinants of erythropoietin release in response to short term, hypobaric hypoxia. *J Appl Physiol* 2002;**92**:2361–7.
20 **Witkowski S**, Karlsen T, Resaland G, *et al*. Optimal altitude for ''living high-training low'' [abstract]. *Med Sci Sports Exerc* 2001;**33**:S292.
21 **Abbrecht PH**, Littell JK. Plasma erythropoietin in men and mice during acclimatization to different altitudes. *J Appl Physiol* 1972;**32**:54–8.
22 **Grover RF**, Bärtsch P. Blood. In: Hornbein TF, Schoene RB, eds. *High altitude – an exploration of human adaptation*. New York: Marcel Dekker, 2001:493–523.
23 **Huff RL**, Lawrence JH, Siri WE, *et al*. Effects of changes in altitude on haemopoietic activity. *Medicine* 1951;**30**:197–217.
24 **Refnaforje C**, Lozano R, Valdivieso S. The polycythaemia of high altitude: iron metabolism and related aspects. *Blood* 1959;**14**:433–55.
25 **Lawrence JF**, Huff RL, Siri W, *et al*. A physiological study in the Peruvian Andes. *Acta Med Scand* 1952;**142**:117–31.
26 **Pugh LGCE**. Blood volume and haemoglobin concentration at altitudes above 18000 feet (5500 m). *J Physiol* 1964;**170**:344–54.
27 **Levine BD**. Intermittent hypoxic training: fact and fantasy. *High Alt Med Biol* 2002;**3**(2):177–93.
28 **Vasquez R**, Villena M. Normal haematological values for healthy persons living at 4000 meters in Bolivia. *High Alt Med Biol* 2001;**2**(2):361–7.
29 **Beall CM**, Brittenham GM, Macuaga F, *et al*. Variation in haemoglobin concentration among samples of high-altitude natives in the Andes and the Himalayas. *Am J Hum Biol* 1990;**2**:639–51.
30 **Beall CM**, Decker MJ, Brittenham GM, *et al*. An Ethiopian pattern of human adaptation to high altitude hypoxia. *Proc Natl Acad Sci U S A* 2002;**99**(26):17215–8.
31 **Beall CM**, Brittenham GM, Strohl KP, *et al*. Hemoglobin concentration of high-altitude Tibetans and Bolivian Aymara. *Am J Phys Anthropol* 1998;**106**:385–400.
32 **Beall CM**, Strohl KP, Blangero J, *et al*. Ventilation and hypoxic ventilatory response of Tibetan and Aymara high altitude natives. *Am J Phys Anthrop* 1997;**104**:427–47.
33 **Severinghaus JW**, Bainton CK, Carcelen C. Respiratory insensitivity to hypoxia in chronically hypoxic man. *Respir Physiol* 1966;**1**:308–34.
34 **Moore LG**, Asmus I, Curran L. Chronic mountain sickness: gender and geographical variation. In: Ohno H, Kobayashi T, Masuyama S, *et al*, eds. *Progress in mountain medicine and high altitude*. Matsumoto, Japan: Press Committee on Mountain Medicine and High Altitude Physiology, 1998:114–9.