

METABOLIC ADJUSTMENT TO HIGH-ALTITUDE HYPOXIA: FROM GENETIC SIGNALS TO PHYSIOLOGICAL IMPLICATIONS

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Short title: Metabolic Adjustment to Hypoxia at Altitude

Abstract

Ascent to high altitude is associated with physiological responses that counter the stress of hypobaric hypoxia by increasing oxygen delivery and by altering tissue oxygen utilisation via metabolic modulation. At the cellular level, the transcriptional response to hypoxia is mediated by the hypoxia inducible factor (HIF) pathway, and results in promotion of glycolytic capacity and suppression of oxidative metabolism. In Tibetan highlanders, gene variants encoding components of the HIF-pathway have undergone selection and are associated with adaptive phenotypic changes, including suppression of erythropoiesis and increased blood lactate levels. In some highland populations, there has also been selection of variants in *PPARA*, encoding peroxisome proliferator-activated receptor α ($PPAR\alpha$), a transcriptional regulator of fatty acid metabolism. In one such population, the Sherpas, lower muscle *PPARA* expression is associated with a decreased capacity for fatty acid oxidation, potentially improving the efficiency of oxygen utilisation. In lowlanders ascending to altitude, a similar suppression of fatty acid oxidation occurs, although the underlying molecular mechanism appears to differ along with the consequences. Unlike lowlanders, Sherpas appear to be protected against oxidative stress and the accumulation of intramuscular lipid intermediates at altitude. Moreover, Sherpas are able to defend muscle ATP and phosphocreatine levels in the face of decreased oxygen delivery, possibly due to suppression of ATP demand pathways. The molecular mechanisms allowing Sherpas to successfully live, work and reproduce at altitude may hold the key to novel therapeutic strategies for the treatment of diseases to which hypoxia is a fundamental contributor.

244 words (250 max)

Abbreviations List

CPT	carnitine palmitoyltransferase
EPO	erythropoietin
FAO	fatty acid oxidation
HIF	hypoxia-inducible factor
ICU	intensive care unit
LDH	lactate dehydrogenase
NO	nitric oxide
PCr	phosphocreatine
PDH	pyruvate dehydrogenase
PDK	pyruvate dehydrogenase kinase
PHD	prolyl hydroxylase
PPAR	peroxisome proliferator-activated receptor
TCA	tricarboxylic acid
VEGF	vascular endothelial growth factor

Introduction

As terrestrial altitude increases, barometric pressure falls whilst the atmospheric proportion of oxygen remains constant at 21%. Accordingly, the partial pressure of oxygen decreases at high altitude, giving rise to hypobaric hypoxia. For humans ascending to altitude, the lower partial pressure of inspired oxygen leads to a reduction in the oxygen content of arterial blood (systemic hypoxaemia) and thence to tissue hypoxia (diminished cellular/mitochondrial oxygen availability).

Hypoxaemia and tissue hypoxia are also seen in a number of diseases and are common consequences of critical illness, arising due to perturbations in the pathway of convective (e.g. ventilatory insufficiency, anaemia, microcirculatory dysfunction) and diffusive (pulmonary or tissue oedema) oxygen delivery [1]. Phenotypic heterogeneity in critically ill patients makes this a challenging population in which to explore responses to hypoxia. Studying responses to hypobaric hypoxia in healthy individuals instead offers a possible paradigm through which mechanisms of pathophysiological importance can be scrutinised in the absence of confounding factors associated with patient characteristics, precipitating disease states or therapeutic intervention [1]. The physiological responses to hypobaric hypoxia are diverse and numerous, and their magnitude (just as for phenotypes such as exercise limitation) exhibit significant inter-individual variation to which genetic variation likely contributes substantially [2].

Research into human physiology at high altitude includes studies of acclimatisation (i.e. the beneficial, time-dependent processes that occur in lowlanders in response to lower partial pressures of inspired oxygen), and of adaptation (i.e. the acquisition of physiological traits resulting from natural selection following sustained habitation at altitude over many generations [3]). Sizeable,

permanent, indigenous human populations have become established over thousands of years in high altitude regions (> 2,500 m) in the Ethiopian Highlands, the Andes and the Tibetan Plateau [4]. Populations in these regions have undergone natural selection, resulting in the appearance of physiological traits that quantifiably enhance oxygen delivery, thereby offsetting the challenging environmental stresses to which these populations are exposed [5]. A growing number of studies have sought to identify the genetic basis of high altitude adaptation [6-16], and collectively suggest that this adaptation has occurred through the alteration of multiple molecular mechanisms that regulate not only oxygen delivery but also oxygen utilisation by cellular metabolism.

Enhanced physical performance at altitude in comparison with lowlanders has been reported in a number of high-altitude populations, and this has been attributed to a decreased metabolic cost of work [17, 18]. In this regard, one highland population that has attracted significant interest is the Sherpa people [19], a Himalayan population of Tibetan descent that migrated to the highlands of Nepal around 500 years ago. Sherpas exhibit remarkable physical performance at extreme altitude [20] and are renowned for their prowess as climbers on the highest Himalayan peaks. There are considerable genetic differences between Sherpas and Tibetans, with divergence between the two groups estimated to have taken place between 3,200 and 11,300 years ago [21]. Sherpa physiology has been subject to investigation for over 50 years, with one of the earliest studies concluding that fundamental mechanisms at the cellular level that improved the efficiency of oxygen utilisation were likely to explain their superior ability to perform under the challenging hypoxic conditions of extreme altitude [22].

Beyond oxygen delivery – metabolic aspects to altitude acclimatisation and adaptation

During acute exposure to hypobaric hypoxia, compensatory mechanisms that increase convective oxygen delivery appear to dominate, with ventilation, cardiac output and haematocrit all increasing in lowlanders as they ascend to altitude [3]. Similarly, in high-altitude populations there has been selection for physiological traits that enhance oxygen flux [5]. It is of note, however, that here the patterns of adaptation differ markedly between highland populations with, for example, Tibetans exhibiting higher resting ventilation rates than Andeans, but lower haematocrits and arterial oxygen contents at a given altitude compared with either Andeans or lowlanders [5]. Exhaled concentrations of the signalling molecule and vasodilator nitric oxide (NO) are elevated in Andeans compared with lowlanders and to an even greater extent in Tibetans [23]. A number of variants in the gene *GCH1* (encoding GTP-cyclohydrolase 1; known to play a role in stabilising NO synthase activity) were recently found to be enhanced in Tibetans, in association with elevated circulating NO levels [12]. Increased NO availability may promote enhanced pulmonary perfusion and afford protection against the pulmonary hypertension experienced by lowlanders at altitude as NO production decreases [24]. Elevated circulating NO metabolites are also associated with enhanced limb blood flow in Tibetans [25], and NO may itself play a role in regulating haematocrit [26] thereby decreasing blood viscosity. Sherpas, meanwhile, have higher sublingual capillary densities and microcirculatory blood flow than lowlanders [27] and Tibetans have greater muscle myoglobin contents [28], further underlining the importance of enhanced oxygen flux as a key facet of adaptation.

Mechanisms influencing oxygen delivery, however, do not fully account for inter-individual differences in performance at altitude [2], and it is increasingly recognised that acclimatisation involves not only changes in oxygen delivery, but also metabolic alterations that modify oxygen

utilisation at the cellular level [29]. Notably, despite a normalisation of arterial oxygen content to sea-level values following acclimatisation [30] performance in lowlanders remains impaired, whilst in Tibetans/Sherpas, selection has favoured a lower oxygen content [5]. Moreover, in fully-acclimatised lowlanders, restoration of arterial oxygen pressures by breathing pure oxygen at altitude does not restore maximal oxygen consumption to sea-level values [31], suggesting a peripheral impairment beyond limitations in oxygen carriage. Similarly, a fall in the myocardial phosphocreatine (PCr) to ATP ratio (an index of cardiac energetic reserve) was seen to persist in acclimatised lowlanders following return to sea level [32], further supporting the concept of metabolic suppression. Of note, however, elevated haematocrit and blood viscosity, following altitude exposure, would increase cardiac work, and this enhanced demand will also likely impact upon cardiac energetics and possibly physical performance at altitude, particularly in the presence of hypoxic pulmonary vasoconstriction.

Mechanistically, a reduction in skeletal muscle mitochondrial content has been observed in lowlanders following prolonged exposure to extreme high altitude during an ascent of Everest (8,848 m) [33, 34]. Similarly, lower muscle mitochondrial contents have been reported in Sherpas [35], lowland-dwelling Tibetans [36] and Andeans [37] compared with lowlander populations, whilst mitochondrial DNA (mtDNA) content was lower in Tibetans [38], indicating that attenuation of cellular oxygen demand is indeed likely to be a beneficial adaptation at altitude. However, no loss of mitochondrial content was seen in lowlander subjects during a simulated ascent to 8,840 m in a decompression chamber [39], nor following ascent to the more moderate altitude of Everest Base Camp (5,300 m) [34]. Nevertheless, in the skeletal muscle of lowlanders exposed to altitudes between 3,000 m and 5,300 m, there are signals of metabolic modulation consistent with a

suppression of oxygen demand, even in the apparent absence of changes in mitochondrial density, including down-regulation of mitochondrial electron transfer system complexes and tricarboxylic acid (TCA) cycle enzymes [29, 40]. Correspondingly, mitochondrial respiratory capacity was suppressed (and coupling efficiency increased) in the muscle of subjects exposed to 3,454 m for 28 days [41]. Such changes are, however, likely to be dependent on duration of exposure as well as altitude, since a similar study by the same group found no change in mitochondrial respiratory function following a shorter exposure of 9-11 days, despite these subjects reaching a higher altitude (4,559 m) [42].

The cellular response to hypoxia is orchestrated by the hypoxia-inducible factor (HIF) family of transcription factors (reviewed in [43]). Under normoxic conditions, the prolyl hydroxylase (PHD) enzymes target the HIF-1 α and HIF-2 α subunits for degradation [44-46], and thus system activity remains low. With low partial pressures of oxygen, however, the HIF- α subunits are stabilised and form heterodimers with the nuclear HIF-1 β subunit. The dimer interacts with hypoxia-response elements in promoter regions to increase the expression of target genes including erythropoietin (*EPO*) and vascular endothelial growth factor A (*VEGFA*), thereby mediating changes in oxygen delivery [43]. The HIF pathway also regulates cellular metabolism, with HIF-1 activation increasing the expression of many glycolytic enzymes [47] as well as pyruvate dehydrogenase kinase 1 (PDK1), which inhibits pyruvate dehydrogenase (PDH) thereby contributing further to the suppression of oxidative metabolism [48, 49]. Evidence supports the selection of genetic variants encoding components of the HIF pathway in Tibetans and Sherpas, including *EPAS1* (encoding HIF-2 α) [6, 11, 13] and *EGLN1* (encoding PHD2) [9]. The Tibetan-enriched variant of *EPAS1* results in lower protein expression and was thus found to be associated both with the lower circulating haemoglobin levels

in Tibetans, but also a suppressed pulmonary vasoconstriction response to hypoxia [14]. Given the role of the HIF-pathway in metabolic modulation, it is likely that the adaptive phenotype in these populations may also include metabolic adjustment in addition to enhancement of oxygen delivery.

Metabolic substrate switching and *PPARA* as a target of interest

Further compelling evidence for metabolic mechanisms of adaptation emerged from a genomic scan in Tibetan highlanders, which highlighted a haplotype of *PPARA* that was positively selected and associated with the phenotype of a lower haematocrit [9]. *PPARA* encodes peroxisome proliferator-activated receptor α ($PPAR\alpha$), a member of the PPAR family of ligand-activated transcription factors that play key roles in regulating cellular energy metabolism. $PPAR\alpha$ is expressed in heart, skeletal muscle and liver, and when activated increases the expression of several genes encoding proteins that control fatty acid metabolism [50, 51]. In Tibetans, the putatively advantageous haplotype of *PPARA* was associated with increased serum levels of non-esterified fatty acids [52], suggesting a possible downregulation of whole-body fatty acid oxidation (FAO), whilst in Sherpas the haplotype was associated with lower skeletal muscle expression of $PPAR\alpha$ and its target, carnitine palmitoyltransferase 1 (*CPT1B*), resulting in decreased mitochondrial FAO capacity [53]. Since the oxygen requirement of ATP synthesis is greater during FAO than glucose oxidation, a switch in substrate preference away from fatty acids represents a further possible mechanism by which cellular oxygen requirements can be lowered in hypoxia. In Sherpas this was also associated with enhanced mitochondrial coupling efficiency (an index of oxidative phosphorylation capacity relative to leak state respiration, during which oxygen is consumed in the absence of ATP synthesis) although this measurement was made in permeabilised muscle fibres *ex vivo*, and evidence of improved

efficiency *in vivo* (e.g. during an exercise challenge) would be important in order to confirm this is associated with a physiological benefit [53].

FAO capacity has also been found to decrease in native lowlanders with sufficient time at altitude, whilst mitochondrial coupling efficiency improves [41, 53], thus the results of acclimatisation appear to resemble the adaptive phenotype of highlanders. Notably though, the underlying molecular mechanisms appear to differ, with only modest elevations in NO production and no change in the abundance of *PPARA* mRNA seen in lowlanders despite a downregulation of its targets [53], suggesting decreased transcriptional activity of PPAR α rather than lowered expression drives the response. A number of other studies have also reported a downregulation of the expression and/or activity of FAO enzymes, many of which are *PPARA* targets, both in human muscle at altitude [34, 40, 53, 54] and in the heart and skeletal muscle of hypoxic rodents [55-57]. Indeed in the hypoxic mouse heart, decreased expression of PPAR α and its targets lowers FAO capacity and represents a vital mechanism to conserve energetics and prevent hypoxic injury [58].

In lowlanders at altitude, this metabolic switch may yet come at a price as long-chain acylcarnitines accumulate in muscle over time [53], suggesting that incomplete FAO leads to the accumulation of potentially-harmful lipid intermediates associated with muscle insulin resistance [59]. In contrast, long-chain acylcarnitine levels remained low in Sherpas' muscle at altitude [53], indicating an alternative mechanism to dispose of fatty acids in the face of lower mitochondrial FAO. The non-mitochondrial pathway of fatty acid ω -oxidation has undergone selection both in the Himalayas and Andes, and intriguingly was found to be the strongest signal of convergent evolution across geographically-separated highland populations [7, 60]. Whilst ω -oxidation is normally a minor

pathway in vertebrates, it increases in importance under conditions where mitochondrial FAO is impaired [61], being viewed as a rescue pathway to prevent lipotoxicity. It is yet to be established, however, whether ω -oxidation flux is altered in skeletal muscle at altitude, either in lowlanders or in adapted highlanders.

A further metabolic switch that improves the efficiency of oxygen utilisation and shows commonality between acclimatising lowlanders and highlander populations is an increased glycolytic flux, with HIF activation known to promote glycolysis [47] and lactate efflux [48, 49] in cells. In Tibetans, a positively-selected haplotype of *EGLN1* was associated with elevated serum lactate levels [52], although notably lactate dehydrogenase (LDH) expression was reported to be downregulated in high-altitude resident Tibetans compared with lowlanders [28]. Sherpas, meanwhile, have elevated muscle lactate dehydrogenase (LDH) activity compared with lowlanders, suggesting an increased capacity for lactate efflux [53] and elevated cardiac glucose uptake [62]. In lowlanders, glucose clearance was enhanced following an oral glucose challenge at altitude, suggesting an increased reliance on glucose metabolism, whilst glycolytic intermediates increased in skeletal muscle [53]. Increased glucose metabolism, particularly glycolysis, is thus a hallmark of both acclimatisation and adaptation to altitude.

A further, recent study reporting signals of high-altitude adaptation in Tibetans highlighted alleles around two genomic loci, namely *EPAS1* and *MTHFR* (encoding methylenetetrahydrofolate reductase) that were associated with circulating haemoglobin, folate and homocysteine levels [16]. Of note, the folate-increasing allele of *MTHFR* was increased in Tibetans, and the authors speculate that this may offset the increased degradation of folate at high-altitude due to increased UV

exposure. Folate is essential for the maturation of red blood cells, but is also known to support lipid metabolism [63]. Moreover, there is emerging evidence that folate deficiency leads to instability in mtDNA transcription, resulting in the altered expression of electron transfer system components and mitochondrial dysfunction (reviewed in [64]). The role of folate availability in the regulation of mitochondrial function and substrate metabolism at altitude deserves further attention.

Comparative and translational aspects

Suppression of cellular oxygen demand and improvement of metabolic efficiency are strategies adopted in extremely hypoxia-tolerant species elsewhere in the animal kingdom [65]. Under truly anoxic conditions, as can be experienced by the crucian carp for example, anaerobic metabolism is critical, whilst under conditions of hypoxia, pathways are favoured that maximise ATP production per mole of oxygen [65]. In such hypoxia-tolerant systems, a hypometabolic response, decreasing oxygen demand rather than increasing supply, is a common strategy [66]. The resulting fall in ATP supply is accompanied by the downregulation of ATP-demand pathways, with ion pumping and protein synthesis suppressed, such that energetic homeostasis is maintained [65]. In Sherpa skeletal muscle, concentrations of ATP and PCr increased at altitude, indicating an improvement in energetic reserve despite a fall in oxygen delivery [53]. This seemingly counter-intuitive finding is likely explained by the activation of hypoxia-sensitive mechanisms that conserve ATP levels by decreasing demand. By way of contrast, in lowlander skeletal muscle, ATP and PCr levels fall at altitude and this loss continues over time, even as the subjects acclimatise [53], suggesting that the suppression of ATP supply in these subjects is not met with a comparable downregulation of ATP demand. In a study of Sherpa cardiac energy metabolism, carried out at sea level, a low PCr/ATP ratio was seen in comparison with lowlanders and this persisted even as the Sherpas acclimatised to sea level [67],

consistent with the notion of an adaptive hypometabolic state and comparable with our data from Sherpa skeletal muscle at low altitude [53]. It would be interesting to see whether cardiac energetics improve in Sherpas as they ascend to altitude, which might be expected if ATP demand pathways are suppressed in a hypoxia-dependent manner.

The advantage of adopting a hypometabolic state in hypoxia may relate to the organism's need to minimise the production of potentially-harmful levels of reactive oxygen species (ROS). ROS can be produced in the cell via a number of mechanisms, including generation as a by-product of oxidative phosphorylation [68] with production increased under hypoxic conditions [69]. In lowlanders ascending to Everest Base Camp, oxidative stress markers were increased in muscle upon arrival, but fell as the subjects acclimatised in conjunction with a suppression of oxidative metabolism, whilst in Sherpas there was no evidence of oxidative stress following ascent [53]. Sherpa muscle also shows lower accumulation of lipofuscin at altitude compared with lowlanders, further supporting the notion of protection against damage [28]. It has been suggested that the suppression of metabolic demand is protective in critically ill patients [70], and data from patients and animal models are consistent with this notion [71, 72]. Speculatively, a better understanding of the strategies adopted by Sherpas to decrease ATP demand and allay oxidative stress may suggest novel therapeutic strategies for patients. Certainly, maximising oxygen delivery has been reported to be ineffective or perhaps even detrimental in some Intensive Care Unit (ICU) patients [73-76], and in the case of oxygen therapy a measured approach may be more effective than simply assuming that more delivery is always better [77]. A more thorough understanding of the dynamic changes to cellular metabolism that occur in critically ill patients is certainly warranted, along with greater

insight into the importance of hypoxia-signalling pathways in the ICU patient and how these pathways interact with other commonly-observed features of critical illness.

Summary/Conclusions

Studies of healthy individuals at altitude have revealed much about the integrated response to sub-acute and sustained hypoxia and the corresponding limits of human tolerance. A spectrum of physiological adaptive changes is often observed in lowlanders at altitude, as are wide inter-individual differences in physical performance. It has been postulated that genetic differences between individuals might explain such variation, and that similar mechanisms might determine clinical outcome in critically ill patients experiencing hypoxia. Moreover, the study of adaptive traits in highlander populations such as the Sherpas, and their genetic basis, could point towards optimal phenotypes for hypoxia tolerance.

Regarding both the processes of acclimatisation and adaptation to altitude it is clear that mechanisms of improved oxygen delivery alone do not provide an adequate explanation, and that regulation of metabolism to alter oxygen utilisation is also a vital component of hypoxia tolerance (Figure 1). In lowlanders, HIF-pathway mediated responses suppress oxidative metabolism and enhance glycolysis, whilst in highland populations the selection of genetic variants support a shift away from fatty acid oxidation and towards more oxygen-efficient metabolism with concomitant downregulation of ATP demand. An improved understanding of the metabolic adjustments that occur in response to hypoxic disease states, in the ICU patient for instance, could indicate if there is therapeutic potential within the molecular mechanisms of adaptation employed by Tibetans and Sherpas at high altitude.

Acknowledgements

The authors would like to thank all of the participants and supporters of the Caudwell Xtreme Everest and Xtreme Everest 2 expeditions, and in particular the subjects who volunteered for these studies.

Declarations of Interest

The authors have no competing interests to declare.

Author Contribution Statement

All authors contributed to the drafting of the manuscript and approved the final submission.

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Figure Legend

Figure 1. Summary of physiological adaptations to high-altitude hypoxia reported or postulated to occur in Tibetans and/or Sherpas, including adaptations to pathways of convective oxygen delivery and cellular oxygen utilisation and energy metabolism. Adaptations that have been postulated to occur are indicated with a question mark. Note that arrows represent differences compared with lowlanders; so, whilst circulating haemoglobin concentrations increase in Tibetans and Sherpas as they ascend to high altitude, at any given altitude these concentrations remain lower than those seen in acclimatised lowlanders, resulting in lower arterial oxygen contents.