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

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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 (PPARα), 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

92 [3]). Sizeable, permanent, indigenous human populations have become established over
93 thousands of years in high altitude regions (> 2,500 m) in the Ethiopian Highlands, the Andes and
94 the Tibetan Plateau [4]. Populations in these regions have undergone natural selection, resulting in
95 the appearance of physiological traits that quantifiably enhance oxygen delivery, thereby
96 offsetting the challenging environmental stresses to which these populations are exposed [5]. A
97 growing number of studies have sought to identify the genetic basis of high altitude adaptation [6-
98 16], and collectively suggest that this adaptation has occurred through the alteration of multiple
99 molecular mechanisms that regulate not only oxygen delivery but also oxygen utilisation by
100 cellular metabolism.
101
102 Enhanced physical performance at altitude in comparison with lowlanders has been reported in a
103 number of high-altitude populations, and this has been attributed to a decreased metabolic cost of
104 work [17, 18]. In this regard, one highland population that has attracted significant interest is the
105 Sherpa people [19], a Himalayan population of Tibetan descent that migrated to the highlands of
106 Nepal around 500 years ago. Sherpas exhibit remarkable physical performance at extreme altitude
107 [20] and are renowned for their prowess as climbers on the highest Himalayan peaks. There are
108 considerable genetic differences between Sherpas and Tibetans, with divergence between the two
109 groups estimated to have taken place between 3,200 and 11,300 years ago [21]. Sherpa physiology
110 has been subject to investigation for over 50 years, with one of the earliest studies concluding that
111 fundamental mechanisms at the cellular level that improved the efficiency of oxygen utilisation
112 were likely to explain their superior ability to perform under the challenging hypoxic conditions of
113 extreme altitude [22].
114

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-1α and HIF-2α 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-1β 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-2α) [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 (PPARα), a member of the PPAR family of ligand-activated transcription factors that play key roles in regulating cellular energy metabolism. PPARα 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 PPARα 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 PPARα 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 PPARα 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

253 [16]. Of note, the folate-increasing allele of *MTHFR* was increased in Tibetans, and the authors
254 speculate that this may offset the increased degradation of folate at high-altitude due to increased
255 UV exposure. Folate is essential for the maturation of red blood cells, but is also known to support
256 lipid metabolism [63]. Moreover, there is emerging evidence that folate deficiency leads to
257 instability in mtDNA transcription, resulting in the altered expression of electron transfer system
258 components and mitochondrial dysfunction (reviewed in [64]). The role of folate availability in the
259 regulation of mitochondrial function and substrate metabolism at altitude deserves further
260 attention.
261
262 **Comparative and translational aspects**
263 Suppression of cellular oxygen demand and improvement of metabolic efficiency are strategies
264 adopted in extremely hypoxia-tolerant species elsewhere in the animal kingdom [65]. Under truly
265 anoxic conditions, as can be experienced by the crucian carp for example, anaerobic metabolism is
266 critical, whilst under conditions of hypoxia, pathways are favoured that maximise ATP production
267 per mole of oxygen [65]. In such hypoxia-tolerant systems, a hypometabolic response, decreasing
268 oxygen demand rather than increasing supply, is a common strategy [66]. The resulting fall in ATP
269 supply is accompanied by the downregulation of ATP-demand pathways, with ion pumping and
270 protein synthesis suppressed, such that energetic homeostasis is maintained [65]. In Sherpa
271 skeletal muscle, concentrations of ATP and PCr increased at altitude, indicating an improvement in
272 energetic reserve despite a fall in oxygen delivery [53]. This seemingly counter-intuitive finding is
273 likely explained by the activation of hypoxia-sensitive mechanisms that conserve ATP levels by
274 decreasing demand. By way of contrast, in lowlander skeletal muscle, ATP and PCr levels fall at
275 altitude and this loss continues over time, even as the subjects acclimatise [53], suggesting that

276 the suppression of ATP supply in these subjects is not met with a comparable downregulation of
277 ATP demand. In a study of Sherpa cardiac energy metabolism, carried out at sea level, a low
278 PCr/ATP ratio was seen in comparison with lowlanders and this persisted even as the Sherpas
279 acclimatised to sea level [67], consistent with the notion of an adaptive hypometabolic state and
280 comparable with our data from Sherpa skeletal muscle at low altitude [53]. It would be interesting
281 to see whether cardiac energetics improve in Sherpas as they ascend to altitude, which might be
282 expected if ATP demand pathways are suppressed in a hypoxia-dependent manner.
283
284 The advantage of adopting a hypometabolic state in hypoxia may relate to the organism's need to
285 minimise the production of potentially-harmful levels of reactive oxygen species (ROS). ROS can be
286 produced in the cell via a number of mechanisms, including generation as a by-product of
287 oxidative phosphorylation [68] with production increased under hypoxic conditions [69]. In
288 lowlanders ascending to Everest Base Camp, oxidative stress markers were increased in muscle
289 upon arrival, but fell as the subjects acclimatised in conjunction with a suppression of oxidative
290 metabolism, whilst in Sherpas there was no evidence of oxidative stress following ascent [53].
291 Sherpa muscle also shows lower accumulation of lipofuscin at altitude compared with lowlanders,
292 further supporting the notion of protection against damage [28]. It has been suggested that the
293 suppression of metabolic demand is protective in critically ill patients [70], and data from patients
294 and animal models are consistent with this notion [71, 72]. Speculatively, a better understanding
295 of the strategies adopted by Sherpas to decrease ATP demand and allay oxidative stress may
296 suggest novel therapeutic strategies for patients. Certainly, maximising oxygen delivery has been
297 reported to be ineffective or perhaps even detrimental in some Intensive Care Unit (ICU) patients
298 [73-76], and in the case of oxygen therapy a measured approach may be more effective than

299 simply assuming that more delivery is always better [77]. A more thorough understanding of the
300 dynamic changes to cellular metabolism that occur in critically ill patients is certainly warranted,
301 along with greater insight into the importance of hypoxia-signalling pathways in the ICU patient
302 and how these pathways interact with other commonly-observed features of critical illness.
303
304 **Summary/Conclusions**
305 Studies of healthy individuals at altitude have revealed much about the integrated response to
306 sub-acute and sustained hypoxia and the corresponding limits of human tolerance. A spectrum of
307 physiological adaptive changes is often observed in lowlanders at altitude, as are wide inter-
308 individual differences in physical performance. It has been postulated that genetic differences
309 between individuals might explain such variation, and that similar mechanisms might determine
310 clinical outcome in critically ill patients experiencing hypoxia. Moreover, the study of adaptive
311 traits in highlander populations such as the Sherpas, and their genetic basis, could point towards
312 optimal phenotypes for hypoxia tolerance.
313
314 Regarding both the processes of acclimatisation and adaptation to altitude it is clear that
315 mechanisms of improved oxygen delivery alone do not provide an adequate explanation, and that
316 regulation of metabolism to alter oxygen utilisation is also a vital component of hypoxia tolerance
317 (Figure 1). In lowlanders, HIF-pathway mediated responses suppress oxidative metabolism and
318 enhance glycolysis, whilst in highland populations the selection of genetic variants support a shift
319 away from fatty acid oxidation and towards more oxygen-efficient metabolism with concomitant
320 downregulation of ATP demand. An improved understanding of the metabolic adjustments that
321 occur in response to hypoxic disease states, in the ICU patient for instance, could indicate if there

322 is therapeutic potential within the molecular mechanisms of adaptation employed by Tibetans and
323 Sherpas at high altitude.

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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.

References

1 Grocott, M., Montgomery, H. and Vercueil, A. (2007) High-altitude physiology and pathophysiology: implications and relevance for intensive care medicine. *Crit Care*. **11**, 203

2 Martin, D. S., Levett, D. Z., Grocott, M. P. and Montgomery, H. E. (2010) Variation in human performance in the hypoxic mountain environment. *Exp Physiol*. **95**, 463-470

3 Peacock, A. J. (1998) ABC of oxygen: oxygen at high altitude. *BMJ*. **317**, 1063-1066

4 Baker, P. T. (1978) The biology of high-altitude peoples. Cambridge University Press, Cambridge

5 Beall, C. M. (2007) Two routes to functional adaptation: Tibetan and Andean high-altitude natives. *Proc Natl Acad Sci USA*. **104 Suppl 1**, 8655-8660

6 Beall, C. M., Cavalleri, G. L., Deng, L., Elston, R. C., Gao, Y., Knight, J., Li, C., Li, J. C., Liang, Y., McCormack, M., Montgomery, H. E., Pan, H., Robbins, P. A., Shianna, K. V., Tam, S. C., Tsering, N., Veeramah, K. R., Wang, W., Wangdui, P., Weale, M. E., Xu, Y., Xu, Z., Yang, L., Zaman, M. J., Zeng, C., Zhang, L., Zhang, X., Zhaxi, P. and Zheng, Y. T. (2010) Natural selection on EPAS1 (HIF2alpha) associated with low hemoglobin concentration in Tibetan highlanders. *Proc Natl Acad Sci USA*.. **107**, 11459-11464

7 Bigham, A., Bauchet, M., Pinto, D., Mao, X., Akey, J. M., Mei, R., Scherer, S. W., Julian, C. G., Wilson, M. J., Lopez Herraez, D., Brutsaert, T., Parra, E. J., Moore, L. G. and Shriver, M. D. (2010) Identifying signatures of natural selection in Tibetan and Andean populations using dense genome scan data. *PLoS Genet*. **6**, e1001116

8 Bigham, A. W. and Lee, F. S. (2014) Human high-altitude adaptation: forward genetics meets the HIF pathway. *Genes Dev*. **28**, 2189-2204

9 Simonson, T. S., Yang, Y., Huff, C. D., Yun, H., Qin, G., Witherspoon, D. J., Bai, Z., Lorenzo, F. R., Xing, J., Jorde, L. B., Prchal, J. T. and Ge, R. (2010) Genetic evidence for high-altitude adaptation in Tibet. *Science*. **329**, 72-75

10 Lorenzo, F. R., Huff, C., Myllymaki, M., Olenchock, B., Swierczek, S., Tashi, T., Gordeuk, V., Wuren, T., Ri-Li, G., McClain, D. A., Khan, T. M., Koul, P. A., Guchhait, P., Salama, M. E., Xing, J., Semenza, G. L., Liberzon, E., Wilson, A., Simonson, T. S., Jorde, L. B., Kaelin, W. G., Jr., Koivunen, P. and Prchal, J. T. (2014) A genetic mechanism for Tibetan high-altitude adaptation. *Nat Genet*. **46**, 951-956

11 Bhandari, S., Zhang, X., Cui, C., Yangla, Liu, L., Ouzhuluobu, Baimakangzhuo, Gonggalanzi, Bai, C., Bianba, Peng, Y., Zhang, H., Xiang, K., Shi, H., Liu, S., Gengdeng, Wu, T., Qi, X. and Su, B. (2017) Sherpas share genetic variations with Tibetans for high-altitude adaptation. *Mol Genet Genomic Med*. **5**, 76-84

12 Guo, Y. B., He, Y. X., Cui, C. Y., Ouzhu, L., Baima, K., Duoji, Z., Deji, Q., Bian, B., Peng, Y., Bai, C. J., Gongga, L., Pan, Y. Y., Qu, Kang, M., Ciren, Y., Baima, Y., Guo, W., Yang, Zhang, H., Zhang, X. M., Zheng, W. S., Xu, S. H., Chen, H., Zhao, S. G., Cai, Y., Liu, S. M., Wu, T. Y., Qi, X. B. and Su, B. (2017) GCH1 plays a role in the high-altitude adaptation of Tibetans. *Zool Res*. **38**, 155-162

13 Hanaoka, M., Droma, Y., Basnyat, B., Ito, M., Kobayashi, N., Katsuyama, Y., Kubo, K. and Ota, M. (2012) Genetic variants in EPAS1 contribute to adaptation to high-altitude hypoxia in Sherpas. *PloS one*. **7**, e50566

14 Peng, Y., Cui, C., He, Y., Ouzhuluobu, Zhang, H., Yang, D., Zhang, Q., Bianbazhuoma, Yang, L., He, Y., Xiang, K., Zhang, X., Bhandari, S., Shi, P., Yangla, Dejiquzong, Baimakangzhuo, Duojizhuoma, Pan, Y., Cirenyangji, Baimayangji, Gonggalanzi, Bai, C., Bianba, Basang, Ciwangsangbu, Xu, S., Chen, H., Liu, S., Wu, T., Qi, X. and Su, B. (2017) Down-Regulation of EPAS1

Transcription and Genetic Adaptation of Tibetans to High-Altitude Hypoxia. *Mol Biol Evol*. **34**, 818-830

15 Peng, Y., Yang, Z., Zhang, H., Cui, C., Qi, X., Luo, X., Tao, X., Wu, T., Ouzhuluobu, Basang, Ciwangsangbu, Danzengduojie, Chen, H., Shi, H. and Su, B. (2011) Genetic variations in Tibetan populations and high-altitude adaptation at the Himalayas. *Mol Biol Evol*. **28**, 1075-1081

16 Yang, J., Jin, Z. B., Chen, J., Huang, X. F., Li, X. M., Liang, Y. B., Mao, J. Y., Chen, X., Zheng, Z., Bakshi, A., Zheng, D. D., Zheng, M. Q., Wray, N. R., Visscher, P. M., Lu, F. and Qu, J. (2017) Genetic signatures of high-altitude adaptation in Tibetans. *Proc Natl Acad Sci USA*.. **114**, 4189-4194

17 Bastien, G. J., Schepens, B., Willems, P. A. and Heglund, N. C. (2005) Energetics of load carrying in Nepalese porters. *Science*. **308**, 1755

18 Minetti, A. E., Formenti, F. and Ardigo, L. P. (2006) Himalayan porter's specialization: metabolic power, economy, efficiency and skill. *Proc Biol Sci*. **273**, 2791-2797

19 Gilbert-Kawai, E., Sheperdigian, A., Adams, T., Mitchell, K., Feelisch, M., Murray, A., Peters, M., Gilbert-Kawai, G., Montgomery, H., Levett, D., Kumar, R., Mythen, M., Grocott, M. and Martin, D. (2015) Design and conduct of Xtreme Everest 2: An observational cohort study of Sherpa and lowlander responses to graduated hypobaric hypoxia. *F1000Res*. **4**, 90

20 Gilbert-Kawai, E. T., Milledge, J. S., Grocott, M. P. and Martin, D. S. (2014) King of the mountains: Tibetan and Sherpa physiological adaptations for life at high altitude. *Physiology*. **29**, 388-402

21 Zhang, C., Lu, Y., Feng, Q., Wang, X., Lou, H., Liu, J., Ning, Z., Yuan, K., Wang, Y., Zhou, Y., Deng, L., Liu, L., Yang, Y., Li, S., Ma, L., Zhang, Z., Jin, L., Su, B., Kang, L. and Xu, S. (2017) Differentiated demographic histories and local adaptations between Sherpas and Tibetans. *Genome Biol*. **18**, 115

22 Lahiri, S. and Milledge, J. S. (1965) Sherpa physiology. *Nature*. **207**, 610-612

23 Beall, C. M., Laskowski, D., Strohl, K. P., Soria, R., Villena, M., Vargas, E., Alarcon, A. M., Gonzales, C. and Erzurum, S. C. (2001) Pulmonary nitric oxide in mountain dwellers. *Nature*. **414**, 411-412

24 Busch, T., Bartsch, P., Pappert, D., Grunig, E., Hildebrandt, W., Elser, H., Falke, K. J. and Swenson, E. R. (2001) Hypoxia decreases exhaled nitric oxide in mountaineers susceptible to high-altitude pulmonary edema. *Am J Respir Crit Care Med*. **163**, 368-373

25 Erzurum, S. C., Ghosh, S., Janocha, A. J., Xu, W., Bauer, S., Bryan, N. S., Tejero, J., Hemann, C., Hille, R., Stuehr, D. J., Feelisch, M. and Beall, C. M. (2007) Higher blood flow and circulating NO products offset high-altitude hypoxia among Tibetans. *Proc Natl Acad Sci USA*.. **104**, 17593-17598

26 Ashmore, T., Fernandez, B. O., Evans, C. E., Huang, Y., Branco-Price, C., Griffin, J. L., Johnson, R. S., Feelisch, M. and Murray, A. J. (2015) Suppression of erythropoiesis by dietary nitrate. *FASEB J*. **29**, 1102-1112

27 Gilbert-Kawai, E., Coppel, J., Court, J., van der Kaaij, J., Vercueil, A., Feelisch, M., Levett, D., Mythen, M., Grocott, M. P., Martin, D. and Xtreme Everest 2 Research, G. (2017) Sublingual microcirculatory blood flow and vessel density in Sherpas at high altitude. *J Applied Physiol*. **122**, 1011-1018

28 Gelfi, C., De Palma, S., Ripamonti, M., Eberini, I., Wait, R., Bajracharya, A., Marconi, C., Schneider, A., Hoppeler, H. and Cerretelli, P. (2004) New aspects of altitude adaptation in Tibetans: a proteomic approach. *FASEB J*. **18**, 612-614

29 Horscroft, J. A. and Murray, A. J. (2014) Skeletal muscle energy metabolism in environmental hypoxia: climbing towards consensus. *Extrem Physiol Med*. **3**, 19

30 Grocott, M. P., Martin, D. S., Levett, D. Z., McMorrow, R., Windsor, J., Montgomery, H. E. and Caudwell Xtreme Everest Research, G. (2009) Arterial blood gases and oxygen content in climbers on Mount Everest. *New Engl J Med*. **360**, 140-149

31 Cerretelli, P. (1976) Limiting factors to oxygen transport on Mount Everest. *J Appl Physiol*. **40**, 658-667

32 Holloway, C. J., Montgomery, H. E., Murray, A. J., Cochlin, L. E., Codreanu, I., Hopwood, N., Johnson, A. W., Rider, O. J., Levett, D. Z., Tyler, D. J., Francis, J. M., Neubauer, S., Grocott, M. P., Clarke, K. and Caudwell Xtreme Everest Research, G. (2011) Cardiac response to hypobaric hypoxia: persistent changes in cardiac mass, function, and energy metabolism after a trek to Mt. Everest Base Camp. *FASEB J*. **25**, 792-796

33 Hoppeler, H., Howald, H. and Cerretelli, P. (1990) Human muscle structure after exposure to extreme altitude. *Experientia*. **46**, 1185-1187

34 Levett, D. Z., Radford, E. J., Menassa, D. A., Graber, E. F., Morash, A. J., Hoppeler, H., Clarke, K., Martin, D. S., Ferguson-Smith, A. C., Montgomery, H. E., Grocott, M. P., Murray, A. J. and Caudwell Xtreme Everest Research, G. (2012) Acclimatization of skeletal muscle mitochondria to high-altitude hypoxia during an ascent of Everest. *FASEB J*. **26**, 1431-1441

35 Kayser, B., Hoppeler, H., Claassen, H. and Cerretelli, P. (1991) Muscle structure and performance capacity of Himalayan Sherpas. *J Appl Physiol*. **70**, 1938-1942

36 Kayser, B., Hoppeler, H., Desplanches, D., Marconi, C., Broers, B. and Cerretelli, P. (1996) Muscle ultrastructure and biochemistry of lowland Tibetans. *J Appl Physiol*. **81**, 419-425

37 Desplanches, D., Hoppeler, H., Tuscher, L., Mayet, M. H., Spielvogel, H., Ferretti, G., Kayser, B., Leuenberger, M., Grunenfelder, A. and Favier, R. (1996) Muscle tissue adaptations of high-altitude natives to training in chronic hypoxia or acute normoxia. *J Appl Physiol*. **81**, 1946-1951

38 Li, Y., Huang, W., Yu, Q., Cheng, Y. T. and Kong, Q. P. (2016) Lower mitochondrial DNA content relates to high-altitude adaptation in Tibetans. *Mitochondrial DNA A DNA Mapp Seq Anal*. **27**, 753-757

39 Green, H. J., Sutton, J. R., Cymerman, A., Young, P. M. and Houston, C. S. (1989) Operation Everest II: adaptations in human skeletal muscle. *J Appl Physiol*. **66**, 2454-2461

40 Levett, D. Z., Vigano, A., Capitanio, D., Vasso, M., De Palma, S., Moriggi, M., Martin, D. S., Murray, A. J., Cerretelli, P., Grocott, M. P. and Gelfi, C. (2015) Changes in muscle proteomics in the course of the Caudwell Research Expedition to Mt. Everest. *Proteomics*. **15**, 160-171

41 Jacobs, R. A., Siebenmann, C., Hug, M., Toigo, M., Meinild, A. K. and Lundby, C. (2012) Twenty-eight days at 3454-m altitude diminishes respiratory capacity but enhances efficiency in human skeletal muscle mitochondria. *FASEB J*. **26**, 5192-5200

42 Jacobs, R. A., Boushel, R., Wright-Paradis, C., Calbet, J. A., Robach, P., Gnaiger, E. and Lundby, C. (2013) Mitochondrial function in human skeletal muscle following high-altitude exposure. *Exp Physiol*. **98**, 245-255

43 Semenza, G. L. (2012) Hypoxia-inducible factors in physiology and medicine. *Cell*. **148**, 399-408

44 Epstein, A. C., Gleadle, J. M., McNeill, L. A., Hewitson, K. S., O'Rourke, J., Mole, D. R., Mukherji, M., Metzen, E., Wilson, M. I., Dhanda, A., Tian, Y. M., Masson, N., Hamilton, D. L., Jaakkola, P., Barstead, R., Hodgkin, J., Maxwell, P. H., Pugh, C. W., Schofield, C. J. and Ratcliffe, P. J. (2001) C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell*. **107**, 43-54

45 Ivan, M., Kondo, K., Yang, H., Kim, W., Valiando, J., Ohh, M., Salic, A., Asara, J. M., Lane, W. S. and Kaelin, W. G., Jr. (2001) HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. *Science*. **292**, 464-468
46 Jaakkola, P., Mole, D. R., Tian, Y. M., Wilson, M. I., Gielbert, J., Gaskell, S. J., von Kriegsheim, A., Hebestreit, H. F., Mukherji, M., Schofield, C. J., Maxwell, P. H., Pugh, C. W. and Ratcliffe, P. J. (2001) Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. *Science*. **292**, 468-472
47 Semenza, G. L., Roth, P. H., Fang, H. M. and Wang, G. L. (1994) Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *J Biol Chem*. **269**, 23757-23763
48 Kim, J. W., Tchernyshyov, I., Semenza, G. L. and Dang, C. V. (2006) HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab*. **3**, 177-185
49 Papandreou, I., Cairns, R. A., Fontana, L., Lim, A. L. and Denko, N. C. (2006) HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell metab*. **3**, 187-197
50 Gulick, T., Cresci, S., Caira, T., Moore, D. D. and Kelly, D. P. (1994) The peroxisome proliferator-activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression. *Proc Natl Acad Sci USA*.. **91**, 11012-11016
51 Gilde, A. J. and Van Bilsen, M. (2003) Peroxisome proliferator-activated receptors (PPARS): regulators of gene expression in heart and skeletal muscle. *Acta Physiol Scand*. **178**, 425-434
52 Ge, R. L., Simonson, T. S., Cooksey, R. C., Tanna, U., Qin, G., Huff, C. D., Witherspoon, D. J., Xing, J., Zhengzhong, B., Prchal, J. T., Jorde, L. B. and McClain, D. A. (2012) Metabolic insight into mechanisms of high-altitude adaptation in Tibetans. *Mol Genet Metab*. **106**, 244-247
53 Horscroft, J. A., Kotwica, A. O., Laner, V., West, J. A., Hennis, P. J., Levett, D. Z. H., Howard, D. J., Fernandez, B. O., Burgess, S. L., Ament, Z., Gilbert-Kawai, E. T., Vercueil, A., Landis, B. D., Mitchell, K., Mythen, M. G., Branco, C., Johnson, R. S., Feelisch, M., Montgomery, H. E., Griffin, J. L., Grocott, M. P. W., Gnaiger, E., Martin, D. S. and Murray, A. J. (2017) Metabolic basis to Sherpa altitude adaptation. *Proc Natl Acad Sci USA*. **114**, 6382-6387
54 Roberts, A. C., Butterfield, G. E., Cymerman, A., Reeves, J. T., Wolfel, E. E. and Brooks, G. A. (1996) Acclimatization to 4,300-m altitude decreases reliance on fat as a substrate. *J Appl Physiol*. **81**, 1762-1771
55 Ashmore, T., Fernandez, B. O., Branco-Price, C., West, J. A., Cowburn, A. S., Heather, L. C., Griffin, J. L., Johnson, R. S., Feelisch, M. and Murray, A. J. (2014) Dietary nitrate increases arginine availability and protects mitochondrial complex I and energetics in the hypoxic rat heart. *J Physiol*. **592**, 4715-4731
56 Ashmore, T., Roberts, L. D., Morash, A. J., Kotwica, A. O., Finnerty, J., West, J. A., Murfitt, S. A., Fernandez, B. O., Branco, C., Cowburn, A. S., Clarke, K., Johnson, R. S., Feelisch, M., Griffin, J. L. and Murray, A. J. (2015) Nitrate enhances skeletal muscle fatty acid oxidation via a nitric oxide-cGMP-PPAR-mediated mechanism. *BMC Biol*. **13**, 110
57 Horscroft, J. A., Burgess, S. L., Hu, Y. and Murray, A. J. (2015) Altered Oxygen Utilisation in Rat Left Ventricle and Soleus after 14 Days, but Not 2 Days, of Environmental Hypoxia. *PloS one*. **10**, e0138564
58 Cole, M. A., Abd Jamil, A. H., Heather, L. C., Murray, A. J., Sutton, E. R., Slingo, M., Sebag-Montefiore, L., Tan, S. C., Aksentijevic, D., Gildea, O. S., Stuckey, D. J., Yeoh, K. K., Carr, C. A., Evans, R. D., Aasum, E., Schofield, C. J., Ratcliffe, P. J., Neubauer, S., Robbins, P. A. and Clarke, K. (2016)

512 On the pivotal role of PPARalpha in adaptation of the heart to hypoxia and why fat in the diet
513 increases hypoxic injury. *Proc Natl Acad Sci USA*. **30**, 2684-2697
514 59 Koves, T. R., Ussher, J. R., Noland, R. C., Slentz, D., Mosedale, M., Ilkayeva, O., Bain, J.,
515 Stevens, R., Dyck, J. R., Newgard, C. B., Lopaschuk, G. D. and Muoio, D. M. (2008) Mitochondrial
516 overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. *Cell*
517 *Metab*. **7**, 45-56
518 60 Foll, M., Gaggiotti, O. E., Daub, J. T., Vatsiou, A. and Excoffier, L. (2014) Widespread signals
519 of convergent adaptation to high altitude in Asia and America. *Am J Hum Genet*. **95**, 394-407
520 61 Wanders, R. J., Komen, J. and Kemp, S. (2011) Fatty acid omega-oxidation as a rescue
521 pathway for fatty acid oxidation disorders in humans. *FEBS J*. **278**, 182-194
522 62 Holden, J. E., Stone, C. K., Clark, C. M., Brown, W. D., Nickles, R. J., Stanley, C. and
523 Hochachka, P. W. (1995) Enhanced cardiac metabolism of plasma glucose in high-altitude natives:
524 adaptation against chronic hypoxia. *J Appl Physiol*. **79**, 222-228
525 63 Fidanza, A. and Audisio, M. (1982) Vitamins and lipid metabolism. *Acta Vitaminol Enzymol*.
526 **4**, 105-114
527 64 Ormazabal, A., Casado, M., Molero-Luis, M., Montoya, J., Rahman, S., Aylett, S. B.,
528 Hargreaves, I., Heales, S. and Artuch, R. (2015) Can folic acid have a role in mitochondrial
529 disorders? *Drug Discov Today*. **20**, 1349-1354
530 65 Hochachka, P. W., Buck, L. T., Doll, C. J. and Land, S. C. (1996) Unifying theory of hypoxia
531 tolerance: molecular/metabolic defense and rescue mechanisms for surviving oxygen lack. *Proc*
532 *Natl Acad Sci USA*. **93**, 9493-9498
533 66 Gorr, T. A. (2017) Hypometabolism as the ultimate defence in stress response: how the
534 comparative approach helps understanding of medically relevant questions. *Acta Physiol (Oxf)*.
535 **219**, 409-440
536 67 Hochachka, P. W., Clark, C. M., Holden, J. E., Stanley, C., Ugurbil, K. and Menon, R. S.
537 (1996) 31P magnetic resonance spectroscopy of the Sherpa heart: a phosphocreatine/adenosine
538 triphosphate signature of metabolic defense against hypobaric hypoxia. *Proc Natl Acad Sci USA*.
539 **93**, 1215-1220
540 68 Giordano, F. J. (2005) Oxygen, oxidative stress, hypoxia, and heart failure. *J Clin Invest*.
541 **115**, 500-508
542 69 Guzy, R. D. and Schumacker, P. T. (2006) Oxygen sensing by mitochondria at complex III:
543 the paradox of increased reactive oxygen species during hypoxia. *Exp Physiol*. **91**, 807-819
544 70 Singer, M., De Santis, V., Vitale, D. and Jeffcoate, W. (2004) Multiorgan failure is an
545 adaptive, endocrine-mediated, metabolic response to overwhelming systemic inflammation.
546 *Lancet*. **364**, 545-548
547 71 Brealey, D., Brand, M., Hargreaves, I., Heales, S., Land, J., Smolenski, R., Davies, N. A.,
548 Cooper, C. E. and Singer, M. (2002) Association between mitochondrial dysfunction and severity
549 and outcome of septic shock. *Lancet*. **360**, 219-223
550 72 Brealey, D., Karyampudi, S., Jacques, T. S., Novelli, M., Stidwill, R., Taylor, V., Smolenski, R.
551 T. and Singer, M. (2004) Mitochondrial dysfunction in a long-term rodent model of sepsis and
552 organ failure. *Am J Physiol Regul Integr Comp Physiol*. **286**, R491-497
553 73 Hayes, M. A., Timmins, A. C., Yau, E. H., Palazzo, M., Hinds, C. J. and Watson, D. (1994)
554 Elevation of systemic oxygen delivery in the treatment of critically ill patients. *New Engl J Med*.
555 **330**, 1717-1722

556 74 Gattinoni, L., Brazzi, L., Pelosi, P., Latini, R., Tognoni, G., Pesenti, A. and Fumagalli, R.
557 (1995) A trial of goal-oriented hemodynamic therapy in critically ill patients. SvO2 Collaborative
558 Group. *New Engl J Med*. **333**, 1025-1032
559 75 Kern, J. W. and Shoemaker, W. C. (2002) Meta-analysis of hemodynamic optimization in
560 high-risk patients. *Crit Care Med*. **30**, 1686-1692
561 76 Poeze, M., Greve, J. W. and Ramsay, G. (2005) Meta-analysis of hemodynamic
562 optimization: relationship to methodological quality. *Crit Care*. **9**, R771-779
563 77 Martin, D. S. and Grocott, M. P. (2013) Oxygen therapy in critical illness: precise control of
564 arterial oxygenation and permissive hypoxemia. *Crit Care Med*. **41**, 423-432

565

566 **Figure Legend**

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