

Beat Schuler, Carsten Lundby and Max Gassmann

Institute of Veterinary Physiology, Vetsuisse Faculty and Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland

HIF-1 and the adaptation of man to high altitude

Abstract

The hypoxia-inducible factor-1 (HIF-1) is a key regulator of oxygen homeostasis. This heterodimeric transcription factor is composed of an oxygen-regulated α -subunit and a constitutively expressed β (or ARNT)-subunit. The activation of HIF-1 is mainly dependent on the hypoxia induced stabilisation of the α -subunit, whereas the β -subunit is independent on the oxygen partial pressure. Until now about 100 target genes, such as erythropoietin (EPO), vascular endothelial growth factor (VEGF), glucose transporter and glycolytic enzymes have been shown to be regulated by HIF-1. HIF-target genes encode proteins that ultimately increase oxygen delivery or allow metabolic adaptation to reduced oxygen availability. As such, HIF-1 is required for a variety of physiological responses on the cellular and systemic level. Here, we discuss the regulation and function of HIF-1 as well as its target genes upon exposure to high altitude.

Key words:

EPO, exercise, VEGF, human, hypoxia, muscle

Zusammenfassung

Der Hypoxie-induzierbare Faktor-1 (HIF-1) übernimmt eine Schlüsselfunktion bei der Regulierung der Sauerstoffhomöostase. HIF-1 ist ein heterodimerer Transkriptionsfaktor, welcher aus einer sauerstoffregulierten α -Untereinheit und einer konstitutiv exprimierten β -Untereinheit besteht. Die Aktivierung von HIF-1 ist hauptsächlich abhängig von der Hypoxie-bedingten Stabilisierung der α -Untereinheit, währenddessen die β -Untereinheit unabhängig des Sauerstoffpartialdruckes exprimiert wird. Bis heute sind gegen 100 HIF-Zielgene, wie Erythropoietin (EPO), vascular endothelial growth factor (VEGF), Glucosetransporter und glycolytische Enzyme, bekannt. Diese Zielgene kodieren Proteine, welche die Sauerstoffzufuhr erhöhen oder die Anpassung des Metabolismus bei reduzierter Sauerstoffverfügbarkeit ermöglichen. Somit ist HIF-1 für eine Reihe von physiologischen Antworten auf zellulärer und systemischer Ebene massgeblich mitverantwortlich. Hier berichten wir über die Regulation und Funktion von HIF-1 sowie der Zielgene bei Exposition in grosser Höhe.

Schweizerische Zeitschrift für «Sportmedizin und Sporttraumatologie» 53 (2), 82–87, 2005

Introduction

Adequate oxygen supply is essential to the aerobic metabolism of most eukaryotic organisms. As a terminal electron acceptor it is used as a substrate for mitochondrial oxidative phosphorylation and thus involved in the production of energy within a cell. Even slight reduction of oxygen availability (hypoxia) seriously impairs this energy metabolism in humans. Oxygen may become limited by anaemia or due to cardiovascular, pulmonary or haematological diseases but also with exercise and high altitude exposure.

While the percentage of oxygen in the atmosphere below 10,000 m above sea level is essentially constant at about 20–21%, the oxygen partial pressure (pO_2) drops rapidly with increased altitude. With a decrease in pO_2 , the inspiratory and the alveolar pO_2 is also been reduced. For instance, at sea level the mean alveolar pO_2 is about 100 mmHg, whereas it drops to about 46 mmHg at an altitude of 5000 m.

Physiological responses to hypoxia occur at the systemic and cellular level. The systemic response is mediated by chemoreceptor stimulation and activation of the central and peripheral nervous system causing changes in overall parameters such as respiration and heart rate [14, 37, 39, 78]. These parameters are well investigated and will not be discussed in this review. The cellular response is mediated by hypoxia-inducible transcription factors that are responsible for oxygen sensing in the cell. The best studied factor of this group is the hypoxia-inducible factor-1 (HIF-1). It is a heterodimeric transcription factor consisting of two subunits, HIF-1 α and HIF-1 β . The latter has previously been identified as the heterodimerization partner of the dioxin receptor/aryl hydrocarbon receptor (AhR) and hence was called AhR nuclear translocator

(ARNT) [16, 24, 76]. Each subunit belongs to the superfamily of the basic helix-loop-helix (bHLH) proteins with a defined characteristic sequence termed PAS (Per-ARNT-Sim)-domain [76]. HIF-1 α and HIF-1 β are ubiquitously expressed in all tissues of many species investigated so far including drosophila, fish, *C. elegans* and mammals [3, 11, 68]. Studies of homozygous HIF-1 α deficient mice resulted in embryonic lethality at midgestation [29, 40, 60]. These embryos show malformation of the brain, heart and vascular system. Obviously, HIF-1 α is essential for normal development. Although transcriptional expression of HIF-1 α has occasionally been reported to be induced during hypoxia, the primary mechanism of regulation has been demonstrated to be posttranslational. HIF-1 α is immediately degraded under normoxia, whereas under hypoxic conditions this process is inhibited enabling the protein to accumulate and heterodimerize with its β -subunit to form a functional HIF-1 complex. Thus the activation of HIF-1 is mainly dependent on the hypoxic induced stabilisation of the α -subunit, while the β -subunit is hypoxia-independent and constantly expressed. It has also been shown that HIF-1 α is not detected above a physiological limit but increases exponentially with declining O_2 whereas HIF-1 β is not affected by the level of oxygenation [34]. The critical limit was about 5% O_2 (35 mmHg) in HeLa cells. Of note, upon exposure of HeLa cells to hypoxia, HIF-1 α protein was induced within two minutes [33]. Upon reoxygenation, HIF-1 α protein levels were already decreased after 4 min [33]. When is HIF-1 α detectable in vivo? Immunohistochemical examination of brain, kidney, liver, heart, and skeletal muscle revealed that HIF-1 α is detectable in mice kept at normoxic conditions and is increased in response to systemic hypoxia [69]. The maximum was reached in the brain, kidney and liver between 1 and 4 hours. The

achievement of maximal HIF-1 α expression depends on the degree and duration of the hypoxic exposure and is different between the organs. In conclusion of this study, there is always a certain HIF-1 α expression necessary in order to produce enough energy through glycolysis that is required for normal cell function.

HIF-1 α protein degradation

The regulation of HIF-1 α is dependent on oxygen availability. Several studies have shown that under physiological conditions HIF-1 α is regulated by protein stabilisation and not at the transcriptional level. HIF-1 α is constantly expressed but rapidly degraded by binding to the von-Hippel-Lindau tumor-suppressor protein (VHL)-mediated ubiquitin-proteasome pathway under normoxia (Fig. 1). It has been demonstrated that VHL is a critical component of the multiprotein E3 ubiquitin ligase complex and responsible for regulating cellular levels of HIF-1 α [52]. This protein directly interacts with the oxygen-dependent degradation domain (ODD) of HIF-1 α [8, 54, 72]. Disruption of the VHL-gene in patients suffering from the von-Hippel-Lindau disease results in a constitutive stabilisation of HIF-1 α and activation of the HIF-1 target genes [64]. Interestingly, these patients frequently develop highly vascularised tumors as a consequence of dysregulation of vascular growth factors.

It is known that the interaction of VHL and the HIF-1 ODD is triggered by prolylhydroxylation of two highly conserved proline residues within the ODD domain [49]. Specific proline hydroxylases require iron and oxygen as co-factor as well as 2-oxoglutarate as co-substrate [28, 30]. Under normoxic conditions, the proline residue is hydroxylated and enables the interaction between VHL with the ODD domain. Under hypoxia, this process is inhibited, preventing the binding of VHL to ODD and the degradation of HIF-1 α .

Another key enzyme controlling the oxygen-dependent modification of HIF-1 α is asparagine hydroxylase that requires the same co-factors and co-substrates as the prolyl hydroxylases [41]. Under normoxia, the conserved asparagines in the carboxyterminal transactivation domain (CAD) are hydroxylated and interfere with the interaction of CAD with the p300 transcription coactivator. The involvement of the prolyl and asparagine hydroxylases in the regulation of HIF-1 α seems to avoid failures of the activation of the hypoxia pathway. How are these hydroxylases regulated?

There are several theories how molecular oxygen regulates the hydroxylases. One of them implicates reactive oxygen species (ROS) such as superoxide and peroxide in the signal transduction process. ROS are produced during mitochondrial respiration. Surprisingly, both an increase and a decrease in ROS production has been found with hypoxic exposure [9, 13, 74]. Therefore, further studies are required to unravel this conflict.

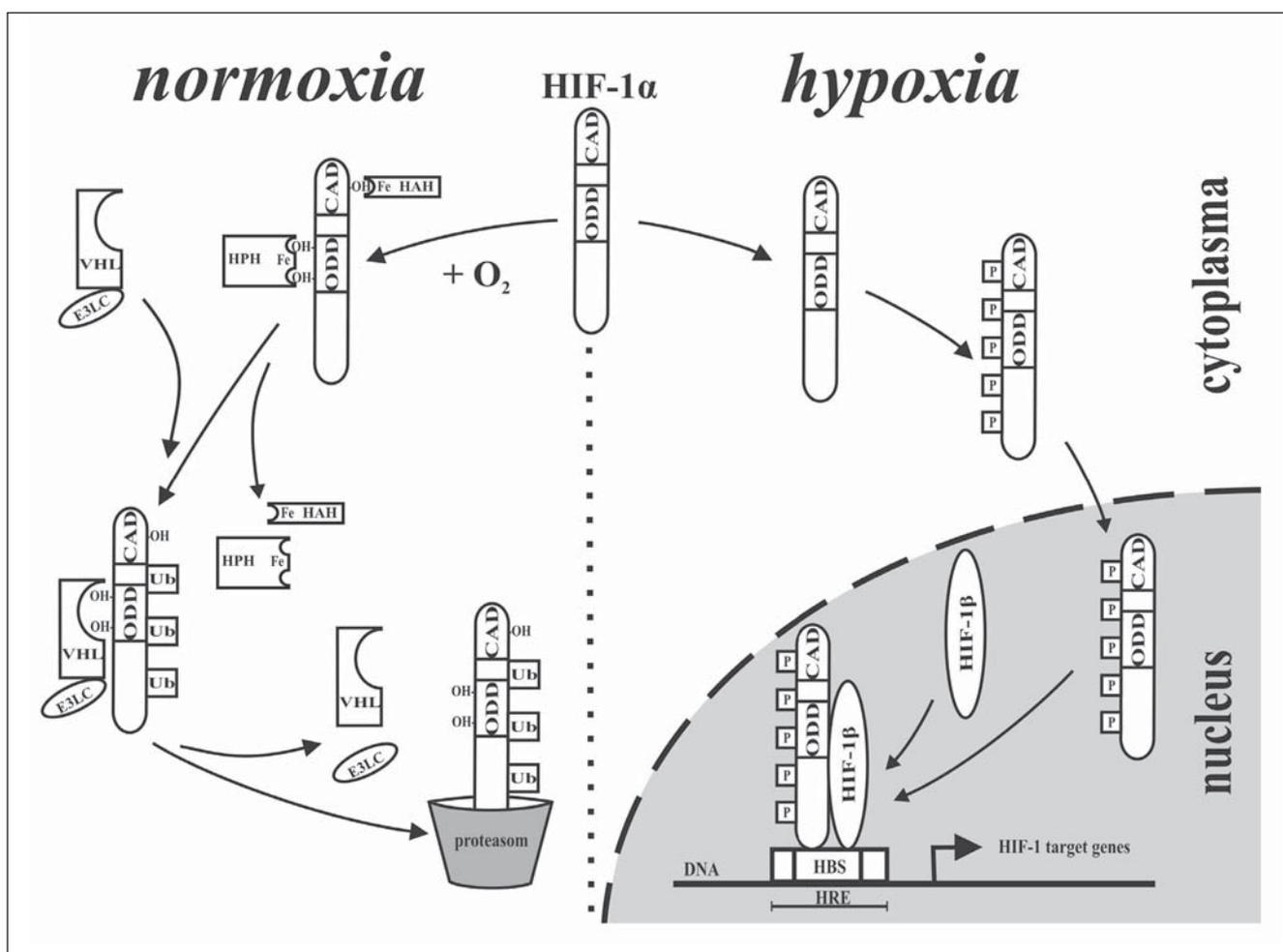


Figure 1: HIF-1 degradation at normoxia and stabilisation at hypoxia.

Under normoxia, HIF-1 α is hydroxylated (-OH) at proline and asparagine residues. The interaction between the hydroxylated proline and asparagine residues and VHL leads finally to proteasomal degradation. Under hypoxia, the hydroxylation of HIF-1 α is inhibited. Thus, HIF-1 α is stabilized and subsequently translocates to the nucleus where it heterodimerizes with HIF-1 β . The resulting HIF-1 complex binds to the HIF-1 binding site present in the hypoxia-response elements. Abbreviations: CAD: carboxyterminal transactivation domain, E3LC: multiprotein E3 ligase complex, HAH: asparagines hydroxylase, HBS: HIF-binding site, HRE: hypoxia-response element, HPH: HIF-1 α prolyl hydroxylase, ODD: oxygen dependent-degradation domain, Ub: ubiquitination, VHL: von-Hippel-Lindau tumor-suppressor protein.

HIF-1 α protein stabilisation

During hypoxic exposure, HIF-1 α stabilizes in the cytoplasm and translocates subsequently to the nucleus (*Fig. 1*) [36]. This translocation is independent of HIF-1 β that is present in the nucleus [7]. After entering the nucleus, HIF-1 α heterodimerizes with HIF-1 β and the resulting HIF-1 complex binds to a specific sequence called the HIF-1 binding site (HBS) present in the hypoxia-response elements (HRE) of all HIF-dependent targets [25]. An increase in nuclear HIF-1 concentration leads to elevated transcriptional activation of the HIF-1 target genes, but there are also other factors which are involved in this process. For instance, any *in vitro* experiment using an inducible system to overexpress HIF-1 α in an oxygen-independent manner has demonstrated that 100-fold overexpressed HIF-1 α translocates to the nucleus under normoxic and hypoxic condition, but neither was the HIF-1 binding capacity nor were the mRNA-levels of the HIF-1 target genes significantly increased [23]. Interestingly, when using a non-inducible system to express HIF-1 α , we observed rapid death of our cells. This observation suggests that high levels of HIF-1 α represent a chronic response to hypoxia that is lethal to the cells. We made a corresponding observation upon generation of transgenic mouse overexpressing HIF-1 α . While several mouse lines show increased levels of human HIF-1 α mRNA, none of them showed elevated HIF-1 α protein levels when compared to wild type controls, even after prolonged exposure to hypoxia (6% O₂ for 4–6h) [BS and MG, unpublished observation].

Are other mechanisms involved in the regulation of the HIF-1 α ? The phosphorylation of HIF-1 α is a mechanism that is essential for a normal HIF-1 function resulting in increased transcription rate of the HIF-1 target genes [58]. Additionally, phosphorylation inhibitors can prevent the upregulation of HIF-1 dependent target genes [6, 27].

Although O₂ induces mainly the stabilisation of HIF-1 α , there is also an O₂-independent HIF-1 α protein stabilisation. It is known that many factors such as interleukin-1 [22], angiotensin II [59], thrombin [17], insulin and insulin-like growth factors [80] induce the accumulation of HIF-1 α under normoxic conditions. Indeed, recent studies have shown that insulin-like growth factor-1 stimulates increased HIF-1 α expression as well as vascular endothelial growth factor (VEGF) secretion in human retinal pigment epithelial cell line [67, 73]. Yet, the mechanisms by which these factors regulate HIF-1 α are poorly understood.

HIF-1 target genes, high altitude and physical exercise

By now, nearly 100 HIF-1 target genes have been identified [42, 79] and the list grows steadily. HIF-1 plays a key role in the regulation of the oxygen-dependent gene expression. These genes are particularly relevant for homeostasis at the cellular and systemic level. An essential response to hypoxia is the increase of the anaerobic glycolysis in order to compensate the energy deficit in the cell. Therefore HIF-1 upregulates the expression of enzymes involved in glucose uptake (glucose transporters) and glycolysis enzymes. In parallel, HIF-1-dependent vascular structure and factors modify the vascular tonus resulting in an improved blood circulation. *Table 1* lists most of the identified HIF-1 target genes. Two of the best studied genes are erythropoietin (EPO) and VEGF.

A systemic response to hypoxia is the upregulation of EPO, a glycoprotein hormone that is produced mainly in kidney but also in brain, liver, lung, spleen, and testis [12, 50, 51, 70]. As a predominant regulator of erythropoiesis derived from the bloodstream, it induces red blood cell production by stimulating the proliferation, differentiation, maturation and preventing apoptosis of the erythrocytic progenitors in the bone marrow. The stimulation of EPO production is either determined by a reduced number of red blood cells (anaemia) or a reduced oxygen saturation of the erythrocyte (hypoxemia). Both conditions require a reduced pO₂-level in the venous blood and tissue. In adults, hypoxia effects a decrease of the intrarenal pO₂ level and this leads to enhance HIF-1 binding ac-

tivity [66] resulting in increased EPO-production in the kidney and finally, in an elevated EPO plasma level. A benefit of an increased EPO level with physical exercise is a higher exercise performance, a cause by a higher O₂ transport capacity to the muscle.

Under normoxia, some studies performed in untrained and trained athletes have demonstrated that submaximal and maximal physical exercise does not influence the EPO plasma level [2, 15, 38, 56, 61]. In contrast, other studies found a slight increase of the EPO level several hours after exercising [57, 63, 65]. Obviously, more studies have to be performed to unravel this aspect. Interestingly, despite the possible unaltered EPO level, the number of reticulocytes is increased upon physical exercise [62, 77]. In all likelihood, the reduced renal blood flow is not the major factor for EPO production during exercise but instead stress hormones such as cortisol and catecholamines are probably more important to regulate red blood cell production [32].

In contrast to physical exercise, an increased EPO plasma level is already detectable 1–2 h after the initiation of hypobaric hypoxia [10]. The peak is reached between 24 and 48 h at a sojourn to moderate (1500–3000 m) and high (> 3000 m) altitude [1, 18, 21, 53]. After this point, the plasma EPO levels decline to nearly pre-exposure levels and may remain slightly elevated when the subjects continue to stay at altitude [21, 48, 53]. Increasing EPO levels is not only detected after an ascent from sea level to altitude, but also when ascending from a moderate altitude to higher altitude. The effect of acute exposure to hypoxia on EPO elevations is maintained after 3 weeks of acclimatization, and even after life long intermittent residency at high altitude [21].

The acclimatization to moderate altitude, when combined with low-altitude training (termed «living high – training low») can improve the exercise performance of athletes at sea level [4, 43]. Levine and coworkers reported that there are two groups of responders. One group termed «responders» displayed a larger increase of plasma EPO concentration at the altitude compared to the second group of «non-responders». The higher EPO concentration found in responders led to increased total red cell volume and VO₂max value at sea level in contrast to the non-responders who showed no alterations. The variation of the increase of the EPO concentration could be explained by individual genetically differences of the «EPO-hypoxic-response» pathway [4]. Very recently, the link of the variability of the genetic EPO mechanism and the individual EPO response to high altitude has been studied [31]. The authors investigated a possible correlation of polymorphic bases linked to the functionally important genes, such as EPO, EPO-receptors, HIF-1 α , pVHL, prolyl hydroxylase genes and others, to individual variation of EPO level during hypoxic exposure to identify the responsible gene(s). However, no convincing association was found. Nevertheless, the impact of other genetic factors can not be excluded. It should be noted that the altitude used was only 2500 m, and one could speculate that as the drive for EPO production becomes greater with increasing altitude, that also the «non EPO responding effect» becomes equally reduced, and eventually diminishes. While the effects of HIF-1 on plasma EPO levels seem straightforward, the effect on muscle tissue is virtually unknown (see below).

It is well known that muscle fibre capillarity is increased with regular endurance exercise. Such an adaptation is advantageous since a given amount of blood will pass the muscle fibers at a slower flow rate, and therefore increase the time for gas and metabolite exchange across the fibre. Thus, an increased capillary density could be a logical adaptive mechanism to chronic hypoxia exposure. HIF-1 α has been recognized to cause an increased expression of at least one important angiogenic factor, VEGF [35].

Under normoxic condition, it is reported that the VEGF mRNA expression correlates positively with the mRNA expression of HIF-1 α and HIF-1 β in resting human muscle [20] and after acute exercise-induced changes in VEGF, HIF-1 α and HIF-1 β mRNA expression [19]. Of note, it is still unknown as whether the modified VEGF mRNA expression is caused by altered HIF-1 expression. Alternatively, one might postulate that similar stimuli influence mRNA transcription and/or stabilization of VEGF and both HIF-1

Adenylate kinase 3 (AK-33)		Intestinal trefoil factor
Adrenomedullin (ADM)		Kreatin 14, 18, 19 (KRT14, 18, 19)
Aldolase A (ALDA)		Lactate dehydrogenase A (LDHA)
Aldolase C (ALDC)		LDL-receptor-related protein (LRP1)
ANF/GPI		Leptin (LEP)
Autocrine mobility factor (AMF/GPI)		LDL-receptor-related protein 1 (LRP1)
α_{1B} -adrenergic receptor (α_{1B} -AR)		Metalloproteinase (MMP2)
Carbonic anhydrase 9 (CA-9)		MIC2
Cathepsin D (CATHD)		Multidrugresistance (MDR1)
Ceruloplasmin		NIP3
c-MET		Nitric oxide synthase 2 (NOS2)
Collagen type V (α 1)		NIX
Cyclin G2		NUR77
Differentiated embryo-chondrocyte expressed gene 1,2 (DEC1,2)		Phosphofructokinase L (PFKL)
Ecto-5'-nucleotidase		Phosphoglyceratekinase 1 (PGK1)
Endocrine-gland-derived VEGF (EG-VEGF)		6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3)
Endoglin (ENG)		Plasminogen-activator inhibitor 1 (PAI1)
Endothelin-1 (ET-1)		Prolyl-4-hydroxylase α
Enolase 1 (ENO1)		Pyruvate kinase (PKM)
Erythropoietin (EPO)		p35srj
ETS-1		RTP801
Ferrochelatase (FECH)	[44]	Transferin
Fibronectin 1 (FN1)		Transferin receptor
Glucose transporter 1,3 (GLUT1,3)		Transforming growth factor- α (TGF- α)
Glyceraldehyde-3-P-dehydrogenase (GAPDH)		Transforming growth factor- β 3 (TGF- β 3)
Haem oxygenase-1 (HO-1)		Transglutaminase 2
Hexokinase 1, 2 (HK1, 2)		Triosephosphate isomerase (TPI)
Inhibitor of differentiation/DNA binding 2 (ID2)	[45]	Urokinase plasminogen activator receptor (UPAR)
Insulin-like growth-factor (IGF2)		Vascular growth factor (VEGF)
Insulin-like growth-factor-binding-protein 1, 2, 3 (IGF-BP1, 2, 3)		Vimentin (VIM)
		WAF-1

Table 1: HIF-1 target genes (adapted from 40, 76 and updated)

subunits. Therefore, more investigations are required to understand this aspect.

Data obtained from hypoxic animals are controversial. An increase in VEGF mRNA was found in rat brain after 6 hours of 10% O₂ (equivalent to about 5500 m) exposure [5], and remained elevated for 14 days, but returned to normoxic levels after 21 days. The same result was found in rat skeletal muscle after 1 h at 6% hypoxia [71]. In contrast, another study reported a surprising attenuation of resting VEGF mRNA after 8 weeks exposure to 12% O₂ in the same muscle and species [55]. Interestingly, it has been shown in some animal studies that capillarisation can be augmented by the formation of new capillaries if the animals are exposed to altitude. In human muscle however, no increase in the formation of new capillaries has been reported even after very long exposure times [46] or following exposures to very high altitudes [26]. Increased muscle fibre capillarisation upon acclimatization has been shown, however, this occurred secondary to a decrease in muscle fibre size [26, 47]. It is possible that the catabolic state associated with gastroenteritis in Himalayan mountaineering [26] and decreased physical activity in hypobaric chambers [47] is the main trigger for the reported muscle atrophy. On the other hand, an increase of the muscle capillary density was demonstrated when high-intensive exercise training is performed during a period of 6 weeks at hypoxia while the rest of the day is spent at normoxic conditions («living low – training high») [75]. The authors reported that the level of the HIF-1 α mRNA increases after training under

hypoxic conditions, independently on the training's intensity. Unexpectedly, an elevated VEGF and phosphofructokinase mRNA level was only found after high-intensive training at hypoxia. This apparent discrepancy on the VEGF mRNA response might be explained by the additive effects of hypoxia and mechanical training stimuli. Of note, metabolic stress, like glucose deprivation, is known to induce VEGF expression. Obviously, these data imply that HIF-1 is involved in the regulation of muscle adaptations after hypoxia training.

Outlook

The role of HIF-1 in healthy humans is far from being well understood. In cell lines, HIF-1 is increased very rapidly, and decreases over time even in a hypoxic milieu. Most probably, a similar response occurs in humans. When exposed to altitude, plasma EPO is increased rapidly, most likely as a consequence of HIF-1 stimulation, where after the plasma EPO concentration decreases toward sea level values, and thus follows a similar pattern as HIF-1 in cell lines. In contrast to the induction of EPO levels in blood, the responses of HIF-1 in the human skeletal muscle are unclear. In most animals, hypoxia leads to angiogenesis most probably induced by augmented VEGF levels. In contrast, human skeletal muscle ultra structure does not respond to hypoxia, but responds when hypoxia is combined with exercise. Thus, it seems that some of the main

responses of HIF-1 obtained from cell lines or animal studies are compatible with human acclimatization to high altitude whereas others are not. Future research on the functional role of HIF-1 in human acclimatization to altitude should focus on the quantification of HIF-1 in muscle, and to what extent the expected elevation alters muscle morphology and increased glucose metabolism.

Acknowledgment

We wish to thank O. Ogunshola and S. Keller for critical reading of the manuscript. This work was supported by the Swiss National Science Foundation and the Benzon Foundation.

Address for correspondence:

Prof. Max Gassmann, DVM, Institute of Veterinary Physiology, Vetsuisse Faculty and Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland, Phone: +41 44 635 8801, E-mail: maxg@access.unizh.ch, Homepage: www.vetphys.unizh.ch

References

- 1 Abbrecht P.H., Littell J.K.: Plasma erythropoietin in men and mice during acclimatization to different altitudes. *J. Appl. Physiol.* 32: 54–58, 1972.
- 2 Berglund B., Birgegard G., Hemmingsson P.: Serum erythropoietin in cross-country skiers. *Med. Sci. Sports Exerc.* 20: 208–209, 1988.
- 3 Bruick R.K., McKnight S.L.: A conserved family of prolyl-4-hydroxylases that modify HIF. *Science* 294: 1337–1340, 2001.
- 4 Chapman R.F., Stray-Gundersen J., Levine B.D.: Individual variation in response to altitude training. *J. Appl. Physiol.* 85: 1448–1456, 1998.
- 5 Chavez J.C., Agani F., Pichiule P., LaManna J.C.: Expression of hypoxia-inducible factor-1 α in the brain of rats during chronic hypoxia. *J. Appl. Physiol.* 89: 1937–1942, 2000.
- 6 Chen E.Y., Mazure N.M., Cooper J.A., Giaccia A.J.: Hypoxia activates a platelet-derived growth factor receptor/phosphatidylinositol 3-kinase/Akt pathway that results in glycogen synthase kinase-3 inactivation. *Cancer Res.* 61: 2429–2433, 2001.
- 7 Chilov D., Camenisch G., Kvietikova I., Ziegler U., Gassmann M., Wenger R.H.: Induction and nuclear translocation of hypoxia-inducible factor-1 (HIF-1): heterodimerization with ARNT is not necessary for nuclear accumulation of HIF-1 α . *J. Cell Sci.* 112 (Pt 8): 1203–1212, 1999.
- 8 Cockman M.E., Masson N., Mole D.R., Jaakkola P., Chang G.W., Clifford S.C., Maher E.R., Pugh C.W., Ratcliffe P.J., Maxwell P.H.: Hypoxia inducible factor- α binding and ubiquitylation by the von Hippel-Lindau tumor suppressor protein. *J. Biol. Chem.* 275: 25733–25741, 2000.
- 9 Duranteau J., Chandel N.S., Kulisz A., Shao Z., Schumacker P.T.: Intracellular signaling by reactive oxygen species during hypoxia in cardiomyocytes. *J. Biol. Chem.* 273: 11619–11624, 1998.
- 10 Eckardt K.U., Bouletier U., Kurtz A., Schopen M., Koller E.A., Bauer C.: Rate of erythropoietin formation in humans in response to acute hypobaric hypoxia. *J. Appl. Physiol.* 66: 1785–1788, 1989.
- 11 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., Ratcliffe P.J.: C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell.* 107: 43–54, 2001.
- 12 Fandrey J., Bunn H.F.: In vivo and in vitro regulation of erythropoietin mRNA: measurement by competitive polymerase chain reaction. *Blood* 81: 617–623, 1993.
- 13 Fandrey J., Frede S., Jelkmann W.: Role of hydrogen peroxide in hypoxia-induced erythropoietin production. *Biochem. J.* 303 (Pt 2): 507–510, 1994.
- 14 Frisncho A.R.: Perspectives on functional adaptation of the high altitude native. *Prog. Clin. Biol. Res.* 136: 383–407, 1983.
- 15 Gareau R., Caron C., Brisson G.R.: Exercise duration and serum erythropoietin level. *Horm. Metab. Res.* 23: 355, 1991.
- 16 Gassmann M., Kvietikova I., Rolfs A., Wenger R.H.: Oxygen- and dioxin-regulated gene expression in mouse hepatoma cells. *Kidney Int.* 51: 567–574, 1997.
- 17 Gorlach A., Diebold I., Schini-Kerth V.B., Berchner-Pfannschmidt U., Roth U., Brandes R.P., Kietzmann T., Busse R.: Thrombin activates the hypoxia-inducible factor-1 signaling pathway in vascular smooth muscle cells: Role of the p22(phox)-containing NADPH oxidase. *Circ. Res.* 89: 47–54, 2001.
- 18 Gunga H.C., Kirsch K., Rocker L., Schobersberger W.: Time course of erythropoietin, triiodothyronine, thyroxine, and thyroid-stimulating hormone at 2,315 m. *J. Appl. Physiol.* 76: 1068–1072, 1994.
- 19 Gustafsson T., Puntchart A., Kaijser L., Jansson E., Sundberg C.J.: Exercise-induced expression of angiogenesis-related transcription and growth factors in human skeletal muscle. *Am. J. Physiol.* 276: H679–685, 1999.
- 20 Gustafsson T., Puntchart A., Sundberg C.J., Jansson E.: Related expression of vascular endothelial growth factor and hypoxia-inducible factor-1 mRNAs in human skeletal muscle. *Acta. Physiol. Scand.* 165: 335–336, 1999.
- 21 Heinicke K., Prommer N., Cajigal J., Viola T., Behn C., Schmidt W.: Long-term exposure to intermittent hypoxia results in increased hemoglobin mass, reduced plasma volume, and elevated erythropoietin plasma levels in man. *Eur. J. Appl. Physiol.* 88: 535–543, 2003.
- 22 Hellwig-Burgel T., Rutkowski K., Metzen E., Fandrey J., Jelkmann W.: Interleukin-1 β and tumor necrosis factor- α stimulate DNA binding of hypoxia-inducible factor-1. *Blood* 94: 1561–1567, 1999.
- 23 Hofer T., Desbaillets I., Hopfl G., Gassmann M., Wenger R.H.: Dissecting hypoxia-dependent and hypoxia-independent steps in the HIF-1 α activation cascade: implications for HIF-1 α gene therapy. *Faseb. J.* 15: 2715–2717, 2001.
- 24 Hoffman E.C., Reyes H., Chu F.F., Sander F., Conley L.H., Brooks B.A., Hankinson O.: Cloning of a factor required for activity of the Ah (dioxin) receptor. *Science* 252: 954–958, 1991.
- 25 Hopfl G., Ogunshola O., Gassmann M.: Hypoxia and high altitude. The molecular response. *Adv. Exp. Med. Biol.* 543: 89–115, 2003.
- 26 Hoppeler H., Kleinert E., Schlegel C., Claassen H., Howald H., Kayar S.R., Cerretelli P.: Morphological adaptations of human skeletal muscle to chronic hypoxia. *Int. J. Sports Med.* 11 Suppl. 1: S3–9, 1990.
- 27 Hur E., Chang K.Y., Lee E., Lee S.K., Park H.: Mitogen-activated protein kinase inhibitor PD98059 blocks the trans-activation but not the stabilization or DNA binding ability of hypoxia-inducible factor-1 α . *Mol. Pharmacol.* 59: 1216–1224, 2001.
- 28 Ivan M., Kondo K., Yang H., Kim W., Valiando J., Ohh M., Salic A., Asara J.M., Lane W.S., Kaelin W.G., Jr.: HIF1 α targeted for VHL-mediated destruction by proline hydroxylation: implications for O₂ sensing. *Science* 292: 464–468, 2001.
- 29 Iyer N.V., Kotch L.E., Agani F., Leung S.W., Laughner E., Wenger R.H., Gassmann M., Gearhart J.D., Lawler A.M., Yu A.Y., Semenza G.L.: Cellular and developmental control of O₂ homeostasis by hypoxia-inducible factor 1 α . *Genes Dev* 12: 149–162, 1998.
- 30 Jaakkola P., Mole D.R., Tian Y.M., Wilson M.I., Gielbert J., Gaskell S.J., Kriegsheim A., Hestreit H.F., Mukherji M., Schofield C.J., Maxwell P.H., Pugh C.W., Ratcliffe P.J.: Targeting of HIF- α to the von Hippel-Lindau ubiquitylation complex by O₂-regulated prolyl hydroxylation. *Science* 292: 468–472, 2001.
- 31 Jedlickova K., Stockton D.W., Chen H., Stray-Gundersen J., Witkowski S., Ri-Li G., Jelinek J., Levine B.D., Prchal J.T.: Search for genetic determinants of individual variability of the erythropoietin response to high altitude. *Blood Cells. Mol. Dis.* 31: 175–182, 2003.
- 32 Jelkmann W.: Erythropoietin. *J. Endocrinol. Invest.* 26: 832–837, 2003.
- 33 Jewell U.R., Kvietikova I., Scheid A., Bauer C., Wenger R.H., Gassmann M.: Induction of HIF-1 α in response to hypoxia is instantaneous. *Faseb. J.* 15: 1312–1314, 2001.
- 34 Jiang B.H., Semenza G.L., Bauer C., Marti H.H.: Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of O₂ tension. *Am. J. Physiol.* 271: C1172–1180, 1996.
- 35 Jin K.L., Mao X.O., Nagayama T., Goldsmith P.C., Greenberg D.A.: Induction of vascular endothelial growth factor and hypoxia-inducible factor-1 α by global ischemia in rat brain. *Neuroscience* 99: 577–585, 2000.
- 36 Kallio P.J., Okamoto K., O'Brien S., Carrero P., Makino Y., Tanaka H., Poellinger L.: Signal transduction in hypoxic cells: inducible nuclear translocation and recruitment of the CBP/p300 coactivator by the hypoxia-inducible factor-1 α . *Embo. J.* 17: 6573–6586, 1998.

- 37 Kellogg R.H.: Oxygen and carbon dioxide in the regulation of respiration. *Fed. Proc.* 36: 1658–1663, 1977.
- 38 Klausen T., Breum L., Fogh-Andersen N., Bennett P., Hippe E.: The effect of short and long duration exercise on serum erythropoietin concentrations. *Eur. J. Appl. Physiol. Occup. Physiol.* 67: 213–217, 1993.
- 39 Koller E.A., Drechsel S., Hess T., Macherel P., Boutellier U.: Effects of atropine and propranolol on the respiratory, circulatory, and ECG responses to high altitude in man. *Eur. J. Appl. Physiol. Occup. Physiol.* 57: 163–172, 1988.
- 40 Kotch L.E., Iyer N.V., Laughner E., Semenza G.L.: Defective vascularization of HIF-1alpha-null embryos is not associated with VEGF deficiency but with mesenchymal cell death. *Dev. Biol.* 209: 254–267, 1999.
- 41 Lando D., Peet D.J., Whelan D.A., Gorman J.J., Whitelaw M.L.: Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. *Science* 295: 858–861, 2002.
- 42 Lee J.W., Bae S.H., Jeong J.W., Kim S.H., Kim K.W.: Hypoxia-inducible factor (HIF-1)alpha: its protein stability and biological functions. *Exp. Mol. Med.* 36: 1–12, 2004.
- 43 Levine B.D., Stray-Gundersen J.: «Living high-training low»: effect of moderate-altitude acclimatization with low-altitude training on performance. *J. Appl. Physiol.* 83: 102–112, 1997.
- 44 Liu Y.L., Ang S.O., Weigent D.A., Prchal J.T., Bloomer J.R.: Regulation of ferrochelatase gene expression by hypoxia. *Life Sci.* 75: 2035–2043, 2004.
- 45 Lofstedt T., Jogi A., Sigvardsson M., Gradin K., Poellinger L., Pahlman S., Axelsson H.: Induction of ID2 expression by hypoxia-inducible factor-1: a role in dedifferentiation of hypoxic neuroblastoma cells. *J. Biol. Chem.* 279: 39223–39231, 2004.
- 46 Lundby C., Pilegaard H., Andersen J.L., van Hall G., Sander M., Calbet J.A.: Acclimatization to 4100 m does not change capillary density or mRNA expression of potential angiogenesis regulatory factors in human skeletal muscle. *J. Exp. Biol.* 207: 3865–3871, 2004.
- 47 MacDougall J.D., Green H.J., Sutton J.R., Coates G., Cymerman A., Young P., Houston C.S.: Operation Everest II: structural adaptations in skeletal muscle in response to extreme simulated altitude. *Acta Physiol. Scand.* 142: 421–427, 1991.
- 48 Mairbaurl H., Schobersberger W., Oelz O., Bartsch P., Eckardt K.U., Bauer C.: Unchanged in vivo P50 at high altitude despite decreased erythrocyte age and elevated 2,3-diphosphoglycerate. *J. Appl. Physiol.* 68: 1186–1194, 1990.
- 49 Masson N., Willam C., Maxwell P.H., Pugh C.W., Ratcliffe P.J.: Independent function of two destruction domains in hypoxia-inducible factor-alpha chains activated by prolyl hydroxylation. *Embo. J.* 20: 5197–5206, 2001.
- 50 Maxwell P.H., Ferguson D.J., Nicholls L.G., Iredale J.P., Pugh C.W., Johnson M.H., Ratcliffe P.J.: Sites of erythropoietin production. *Kidney Int.* 51: 393–401, 1997.
- 51 Maxwell P.H., Osmond M.K., Pugh C.W., Heryet A., Nicholls L.G., Tan C.C., Doe B.G., Ferguson D.J., Johnson M.H., Ratcliffe P.J.: Identification of the renal erythropoietin-producing cells using transgenic mice. *Kidney Int.* 44: 1149–1162, 1993.
- 52 Maxwell P.H., Wiesener M.S., Chang G.W., Clifford S.C., Vaux E.C., Cockman M.E., Wykoff C.C., Pugh C.W., Maher E.R., Ratcliffe P.J.: The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* 399: 271–275, 1999.
- 53 Milledge J.S., Cotes P.M.: Serum erythropoietin in humans at high altitude and its relation to plasma renin. *J. Appl. Physiol.* 59: 360–364, 1985.
- 54 Ohh M., Park C.W., Ivan M., Hoffman M.A., Kim T.Y., Huang L.E., Pavletich N., Chau V., Kaelin W.G.: Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. *Nat. Cell. Biol.* 2: 423–427, 2000.
- 55 Olfert I.M., Breen E.C., Mathieu-Costello O., Wagner P.D.: Chronic hypoxia attenuates resting and exercise-induced VEGF, flt-1, and flk-1 mRNA levels in skeletal muscle. *J. Appl. Physiol.* 90: 1532–1538, 2001.
- 56 Ricci G., Masotti M., De Paoli Vitali E., Vedovato M., Zanotti G.: Effects of a mixed physical activity (biathlon) on haematologic parameters, red cell 2,3-DPG and creatine, serum erythropoietin, urinary enzymes and microalbumin. *Eur. J. Haematol.* 45: 178–179, 1990.
- 57 Ricci G., Masotti M., De Paoli Vitali E., Vedovato M., Zanotti G.: Effects of exercise on haematologic parameters, serum iron, serum ferritin, red cell 2,3-diphosphoglycerate and creatine contents, and serum erythropoietin in long-distance runners during basal training. *Acta Haematol.* 80: 95–98, 1988.
- 58 Richard D.E., Berra E., Gothie E., Roux D., Pouyssegur J.: p42/p44 mitogen-activated protein kinases phosphorylate hypoxia-inducible factor 1alpha (HIF-1alpha) and enhance the transcriptional activity of HIF-1. *J. Biol. Chem.* 274: 32631–32637, 1999.
- 59 Richard D.E., Berra E., Pouyssegur J.: Nonhypoxic pathway mediates the induction of hypoxia-inducible factor 1alpha in vascular smooth muscle cells. *J. Biol. Chem.* 275: 26765–26771, 2000.
- 60 Ryan H.E., Lo J., Johnson R.S.: HIF-1 alpha is required for solid tumor formation and embryonic vascularization. *Embo. J.* 17: 3005–3015, 1998.
- 61 Schmidt W., Eckardt K.U., Hilgendorf A., Strauch S., Bauer C.: Effects of maximal and submaximal exercise under normoxic and hypoxic conditions on serum erythropoietin level. *Int. J. Sports Med.* 12: 457–461, 1991.
- 62 Schmidt W., Maassen N., Trost F., Boning D.: Training induced effects on blood volume, erythrocyte turnover and haemoglobin oxygen binding properties. *Eur. J. Appl. Physiol. Occup. Physiol.* 57: 490–498, 1988.
- 63 Schobersberger W., Hobisch-Hagen P., Fries D., Wiedermann F., Rieder-Scharinger J., Villiger B., Frey W., Herold M., Fuchs D., Jelkmann W.: Increase in immune activation, vascular endothelial growth factor and erythropoietin after an ultramarathon run at moderate altitude. *Immunobiology* 201: 611–620, 2000.
- 64 Schumacker P.T.: Hypoxia, anoxia, and O₂ sensing: the search continues. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 283: L918–921, 2002.
- 65 Schwandt H.J., Heyduck B., Gunga H.C., Rocker L.: Influence of prolonged physical exercise on the erythropoietin concentration in blood. *Eur. J. Appl. Physiol. Occup. Physiol.* 63: 463–466, 1991.
- 66 Semenza G.L., Wang G.L.: A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol. Cell. Biol.* 12: 5447–5454, 1992.
- 67 Slomiany M.G., Rosenzweig S.A.: IGF-1-induced VEGF and IGFBP-3 secretion correlates with increased HIF-1 alpha expression and activity in retinal pigment epithelial cell line D407. *Invest. Ophthalmol. Vis. Sci.* 45: 2838–2847, 2004.
- 68 Soitamo A.J., Rabergh C.M., Gassmann M., Sistonen L., Nikinmaa M.: Characterization of a hypoxia-inducible factor (HIF-1alpha) from rainbow trout. Accumulation of protein occurs at normal venous oxygen tension. *J. Biol. Chem.* 276: 19699–19705, 2001.
- 69 Stroka D.M., Burkhardt T., Desbaillets I., Wenger R.H., Neil D.A., Bauer C., Gassmann M., Candinas D.: HIF-1 is expressed in normoxic tissue and displays an organ-specific regulation under systemic hypoxia. *Faseb J.* 15: 2445–2453, 2001.
- 70 Tan C.C., Eckardt K.U., Firth J.D., Ratcliffe P.J.: Feedback modulation of renal and hepatic erythropoietin mRNA in response to graded anemia and hypoxia. *Am. J. Physiol.* 263: F474–481, 1992.
- 71 Tang K., Breen E.C., Wagner H., Brutsaert T.D., Gassmann M., Wagner P.D.: HIF and VEGF relationships in response to hypoxia and sciatic nerve stimulation in rat gastrocnemius. *Respir. Physiol. Neurobiol.* 144: 71–80, 2004.
- 72 Tanimoto K., Makino Y., Pereira T., Poellinger L.: Mechanism of regulation of the hypoxia-inducible factor-1 alpha by the von Hippel-Lindau tumor suppressor protein. *Embo. J.* 19: 4298–4309, 2000.
- 73 Treins C., Giorgetti-Peraldi S., Mardaca J., Monthouel-Kartmann M.N., Van Obberghen E.: Regulation of HIF-1 activity and expression of HIF hydroxylases in response to IGF-1. *Mol. Endocrinol.* 2005.
- 74 Vanden Hoek T.L., Becker L.B., Shao Z., Li C., Schumacker P.T.: Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. *J. Biol. Chem.* 273: 18092–18098, 1998.
- 75 Vogt M., Puntschart A., Geiser J., Zuleger C., Billeter R., Hoppeler H.: Molecular adaptations in human skeletal muscle to endurance training under simulated hypoxic conditions. *J. Appl. Physiol.* 91: 173–182, 2001.
- 76 Wang G.L., Jiang B.H., Rue E.A., Semenza G.L.: Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension. *Proc. Natl. Acad. Sci. USA* 92: 5510–5514, 1995.
- 77 Weight L.M., Byrne M.J., Jacobs P.: Haemolytic effects of exercise. *Clin. Sci. (Lond)* 81: 147–152, 1991.
- 78 West J.B.: Respiratory and circulatory control at high altitudes. *J. Exp. Biol.* 100: 147–157, 1982.
- 79 Yeo E.J., Chun Y.S., Park J.W.: New anticancer strategies targeting HIF-1. *Biochem. Pharmacol.* 68: 1061–1069, 2004.
- 80 Zelzer E., Levy Y., Kahana C., Shilo B.Z., Rubinstein M., Cohen B.: Insulin induces transcription of target genes through the hypoxia-inducible factor HIF-1alpha/ARNT. *Embo. J.* 17: 5085–5094, 1998.