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Adaptation of iron requirement to hypoxic conditions at high altitude

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Gassmann M, Muckenthaler MU. Adaptation of iron requirement to hypoxic conditions at high altitude. J Appl Physiol 119: 1432–1440, 2015. First published July 16, 2015; doi:10.1152/japplphysiol.00248.2015.—Adequate acclimatization time to enable adjustment to hypoxic conditions is one of the most important aspects for mountaineers ascending to high altitude. Accordingly, most reviews emphasize mechanisms that cope with reduced oxygen supply. However, during sojourns to high altitude adjustment to elevated iron demand is equally critical. Thus in this review we focus on the interaction between oxygen and iron homeostasis. We review the role of iron 1) in the oxygen sensing process and erythropoietin (Epo) synthesis, 2) in gene expression control mediated by the hypoxia-inducible factor-2 (HIF-2), and 3) as an oxygen carrier in hemoglobin, myoglobin, and cytochromes. The blood hormone Epo that is abundantly expressed by the kidney under hypoxic conditions stimulates erythropoiesis in the bone marrow, a process requiring high iron levels. To ensure that sufficient iron is provided, Epo-controlled erythroferrone that is expressed in erythroid precursor cells acts in the liver to reduce expression of the iron hormone hepcidin. Consequently, suppression of hepcidin allows for elevated iron release from storage organs and enhanced absorption of dietary iron by enterocytes. As recently observed in sojourners at high altitude, however, iron uptake may be hampered by reduced appetite and gastrointestinal bleeding. Reduced iron availability, as observed in a hypoxic mountaineer, enhances hypoxia-induced pulmonary hypertension and may contribute to other hypoxia-related diseases. Overall, adequate systemic iron availability is an important prerequisite to adjust to high-altitude hypoxia and may have additional implications for disease-related hypoxic conditions.

low oxygen; prolyl hydroxylase; PHD2; hypoxia-inducible factor; erythropoietin; erythroferron; hepcidin; ferroportin; transferrin; iron homeostasis; oxygen sensor; iron sensor; pulmonary hypertension; HAPE; mountaineer

Iron and oxygen are both essential for life. The regulatory mechanisms that maintain their homeostasis influence each other in a tightly coordinated manner. In excess, however, their interaction generates toxic compounds, especially reactive oxygen species. Before delving into the topic, we summarize some facts about iron and oxygen. When do we face hypoxia? While the oxygen concentration in atmospheric air is always about 21%, at high altitude the oxygen partial pressure declines, leading to systemic hypoxia. But hypoxic conditions can also be reached at sea level as exemplified by an exercising athlete whose body’s oxygen supply does not meet its demand. As a consequence, cells need to respond very fast to reduced oxygenation in an organ-specific manner (78). How much iron does the body need? Iron is a key element in iron-containing proteins and as such is crucially involved in a wide variety of pathways. These include oxygen transport (hemoglobin) and storage (myoglobin), the mitochondrial electron transport chain (a critical requirement for energy production), as well as DNA replication and cell proliferation (10, 77). Most iron is required in erythrocytes for oxygen transport (1,800 mg) and in the muscle’s myoglobin (300 mg), while ~1000 mg of iron are stored in the liver. Only 3 mg circulate in the serum bound to transferrin to provide iron to most cell types (30). Interestingly, there is no regulated pathway for iron excretion. Iron that is lost either by desquamation of cells or bleeding is compensated for by absorption of dietary iron by duodenal enterocytes (about 1–2 mg/day). Most iron required for erythropoiesis is provided by reticuloendothelial macrophages (Fig. 1) that recycle 20–25 mg of iron per day from senescent erythrocytes (31). As for oxygen, a well-controlled balance in iron levels is
required to fulfill the body’s demand. Please note that within this short review we cannot cover the entire impact of iron on the body, but we will focus on some aspects that are important under acute hypoxic exposure, as observed in the ascending mountaineer. We will discuss the critical role of iron in sensing hypoxia and in gene expression under hypoxic conditions, and the coordinated regulation of iron and oxygen homeostasis.

Iron’s Crucial Role in Sensing Hypoxia

The starting point of high-altitude expeditions is commonly over 3,000 m above sea level and is often reached by plane, car, or gondola. Most mountaineers taking these routes will experience the burden of not having taken the time to acclimatize at lower altitudes. Indeed, exposure of a lowlander to 1,500 m will induce the body’s response to hypoxia. Note that the cabin air pressure of a commercial airplane flying at around 10,000 m is usually set to mimic the oxygenation occurring at altitudes of 1,700 to 2,500 m (18, 72). What is our first response to hypoxia? The fall in atmospheric pressure hampers oxygen uptake by the alveolae, which causes arterial hypoxemia. To cope immediately with this threatening condition we increase ventilation. At the same time peritubular fibroblasts in the renal cortex (47) synthesize the blood hormone erythropoietin (Epo) that via the circulation reaches the bone marrow and promotes red blood cell maturation (16). Reduced oxygenation stabilizes the \( \alpha \) subunit of the hypoxia-inducible factor-2 (HIF-2\( \alpha \)) that heterodimerizes with its partner aryl hydrocarbon receptor nuclear translocator (ARNT, also termed HIF-\( \beta \)) to enhance erythropoietin (Epo) transcription. A second iron-dependent process adjusts Epo levels to iron availability: HIF-2\( \alpha \) contains an iron-responsive element (IRE) in its 5' untranslated region. Under iron-deficient conditions, when hemoglobin synthesis is reduced, this RNA structure binds to the iron regulatory protein-1 (IRP1) to inhibit HIF-2\( \alpha \) translation. In other words, these mechanisms ensure that Epo synthesis is adjusted to iron availability. Once Epo reaches the bone marrow it promotes red blood cell maturation and proliferation, a process that consumes high amounts of iron. To make sure that sufficient iron is provided systemically, hypoxia-induced soluble factors, such as the Epo-controlled erythroferrone (ErFe) or the growth differentiation factor 15 (GDF15) that are both expressed in erythroid precursor cells, as well as the platelet-derived growth factor BB (PDGF-BB) reach the liver where they reduce expression of hepcidin, the iron hormone that binds the cellular iron exporter ferroportin (Fpn) leading to its internalization and degradation. Thus suppression of hepcidin allows both elevated iron release from storage organs including macrophages and enhanced absorption of dietary iron by enterocytes. In addition, tissue hypoxia or iron deficiency further augments dietary iron absorption in the intestine. Similar to the situation in the kidney, these conditions stabilize the \( \alpha \) subunit of HIF-2\( \alpha \) that stimulates transcription of proteins that control iron absorption: the ferrireductase (dcytb), the apical divalent metal transporter-1 (DMT-1), and the iron exporter Fpn. The iron released from macrophages and duodenal enterocytes is transported bound to transferrin (Tf) ultimately satisfy the iron requirements of erythropoiesis in the bone marrow. For further details see text. CFU-E, colony-forming unit-erythroid; HRE, hypoxia response element.

Fig. 1. The role of iron in oxygen homeostasis. Upon ascent to high altitude, a fall in atmospheric pressure inhibits oxygen uptake by the alveolae and causes arterial hypoxemia. Systemic oxygen levels are monitored by prolyl hydroxylases (PHDs, exemplified by PHD2) in peritubular fibroblasts present in the renal cortex. Iron is a critical cofactor for these dioxygenases. Thus reduced oxygen or iron levels suppress the activity of PHD2 and cause stabilization of the \( \alpha \) subunit of the hypoxia-inducible factor-2 (HIF-2\( \alpha \)) that heterodimerizes with its partner aryl hydrocarbon receptor nuclear translocator (ARNT, also termed HIF-\( \beta \)) to enhance erythropoietin (Epo) transcription. A second iron-dependent process adjusts Epo levels to iron availability: HIF-2\( \alpha \) contains an iron-responsive element (IRE) in its 5' untranslated region. Under iron-deficient conditions, when hemoglobin synthesis is reduced, this RNA structure binds to the iron regulatory protein-1 (IRP1) to inhibit HIF-2\( \alpha \) translation. In other words, these mechanisms ensure that Epo synthesis is adjusted to iron availability. Once Epo reaches the bone marrow it promotes red blood cell maturation and proliferation, a process that consumes high amounts of iron. To make sure that sufficient iron is provided systemically, hypoxia-induced soluble factors, such as the Epo-controlled erythroferrone (ErFe) or the growth differentiation factor 15 (GDF15) that are both expressed in erythroid precursor cells, as well as the platelet-derived growth factor BB (PDGF-BB) reach the liver where they reduce expression of hepcidin, the iron hormone that binds the cellular iron exporter ferroportin (Fpn) leading to its internalization and degradation. Thus suppression of hepcidin allows both elevated iron release from storage organs including macrophages and enhanced absorption of dietary iron by enterocytes. In addition, tissue hypoxia or iron deficiency further augments dietary iron absorption in the intestine. Similar to the situation in the kidney, these conditions stabilize the \( \alpha \) subunit of HIF-2\( \alpha \) that stimulates transcription of proteins that control iron absorption: the ferrireductase (dcytb), the apical divalent metal transporter-1 (DMT-1), and the iron exporter Fpn. The iron released from macrophages and duodenal enterocytes is transported bound to transferrin (Tf) ultimately satisfy the iron requirements of erythropoiesis in the bone marrow. For further details see text. CFU-E, colony-forming unit-erythroid; HRE, hypoxia response element.
of our body: our cells constantly synthesize HIFs as a response to cope with hypoxic exposure in every cell and as a consequence the HIFs are stabilized. It is intriguing to realize that evolution has come up with a very efficient but expensive mechanism to stabilize HIFs. This synthesis is energetically a burden but allows the cell to instantaneously stabilize HIFs when oxygen supply is low (32). This enables the hypoxic cell to respond in an accelerated manner compared with the usual slow transcription/translation process.

Increasing Ventilation at High Altitude

A fast way to reach high altitude is by car—driving to very high passes such as Ticlio in Peru (4,843 m) or Khardung La in Ladakh, India (5,359 m)—or by gondola to Klein Matterhorn in the Swiss Alps (3,883 m). When leaving the gondola and being immediately faced with the lack of oxygen, it is little solace to know that in about 8–10 days more erythrocytes will be readily available to compensate for reduced oxygen availability (see below). Thus the body initially responds to the reduced oxygen partial pressure by enhancing ventilation, a process known as the hypoxic ventilatory response. Interestingly, this process is partially regulated by Epo (25). It is worth mentioning here that (functional) Epo receptors (EpoR) are not exclusively expressed in erythropoietic cells but are detected in a wide variety of other cell types such as cerebral cells (24, 26, 45). Accordingly, serum Epo seems to bind the EpoR present in the carotid bodies that in turn forward this information via the carotid sinus nerve to the respiratory center of the brain stem (74, 75). In addition, Epo is synthesized in the brain in response to hypoxia and binds to its receptor located in brain stem neurons of the central ventilatory center. Thus Epo:EpoR interactions in the carotid body and brain stem increase ventilation when our body is exposed to hypoxia. But how is iron involved here? Most probably, Epo’s upregulation in the brain is mediated by HIF-2 that in analogy to the renal situation is controlled by PHD2 in an iron-dependent manner (see above).

Iron-dependent Expression of Hypoxia-regulated Genes

Cellular iron levels are balanced by the iron regulatory protein (IRP) and iron response element (IRE) system. Two homologous IRPs (IRP1 and IRP2) sense cellular iron levels by distinct mechanisms. Under iron-replete conditions, a cubane iron-sulfur [4Fe-4S] cluster assembles in IRP1, preventing IRE binding of IRP1. During iron deficiency, however, IRP1 binds to IREs as an apoprotein. In contrast, IRP2 does not contain an iron-sulfur cluster and is regulated by iron via proteasome degradation. IRPs bind to RNA stem loop structures (IREs) located in untranslated regions of genes involved in iron uptake, export, storage, and utilization to control their expression at the posttranscriptional level. IRP target genes are further involved in cellular functions not immediately related to iron metabolism (10, 39, 73). A key target gene of IRP1 is HIF-2α, that harbors an IRE within its 5′-UTR, that upon binding IRP1 blocks HIF-2α translation (49, 66) (Fig. 1). IRP1-deficient mice that lack this repressor of HIF-2α translation show derepressed HIF-2α translation in the kidney, elevated Epo serum levels, and a marked transient polycythemia (4, 27). It is remarkable, however, that mutations within the HIF-2α IRE have been excluded as a frequent cause of congenital secondary erythrocytosis/polycythaemia (58). To further strengthen the link between iron and oxygen homeostasis, IRP1 is mainly active when cellular iron levels are low but oxygen levels are sufficient. Hypoxic conditions, however, override the signal generated by low iron and markedly reduce IRP1 activity, allowing for increased HIF-2α translation (12). It should be mentioned that the RNA-binding activity of IRP2 is elevated when facing both low-iron and low-oxygen conditions (see below). Mice deficient for IRP2 show altered body iron distribution and microcytosis (22). Thus the two major control systems of cellular iron homeostasis (IRE/IRP) and oxygen levels (HIF) tightly interact at the molecular level to control iron and oxygen homeostasis.

Control of Dietary Iron Uptake and Macrophage Iron Recycling in Hypoxic Conditions

Complex interactions of the oxygen/iron sensing systems ensure tight regulation of Epo levels in the kidney to adjust the extent of erythropoiesis to iron availability. While the IRP-mediated control of HIF-2α levels is expected to be operational in the kidney where most Epo synthesis occurs, the same mechanism will work in all those tissues where HIF-2-mediated expression of oxygen-dependent genes occurs (Fig. 1). An often neglected but important organ for an ascending mountaineer is the intestine. There, HIF-2 coordinates dietary iron absorption by regulating the transcription of key factors involved in iron uptake (43). These include the ferrireductase dcytB that converts ferric (Fe3+) to ferrous (Fe2+) iron, the divalent metal transporter 1 (DMT1) that imports ferrous iron from the diet into the duodenal enterocyte, and the iron exporter ferroportin (Fpn) that transports iron out of the duodenal enterocyte into the blood stream (reviewed in Ref. 69) (Fig. 1). Consistent with the negative regulation of the expression of duodenal HIF-2 by IRP1, expression of the duodenal iron uptake machinery is elevated in homozygous irp1-deficient mice because of increased HIF-2α levels (4). Apart from their HIF-2-mediated transcriptional control, the expression of both DMT-1 and Fpn is regulated in an iron-dependent manner. These two genes also contain IREs in their transcripts and thus are prone to iron-dependent IRE/IRP-mediated control (reviewed in Ref. 49). While HIF-2α translation is mainly con-
trolled by IRP1, gene expression of the dietary iron transporters DMT-1 and Fpn is additionally regulated by IRP2 (70).

Interestingly, IRP1 protein levels do not change in hypoxic conditions while the abundance of IRP2 proteins is elevated when oxygen is limited (29, 87).

In summary, cellular iron and oxygen homeostasis are tightly interconnected in that the activities of the corresponding sensors, the IRPs and the HIFs, are controlled by iron and oxygen availability. In addition, expression of genes that regulate appropriate cellular and systemic iron levels is maintained by interaction of the iron and oxygen control systems, which ultimately determine the extent of erythropoiesis as well as the rate of intestinal iron absorption.

Most iron utilized for erythropoiesis is recycled from damaged erythrocytes by tissue macrophages. The heme moiety is catabolized by heme oxygenases resulting in iron release and subsequent iron export into the circulation (31). Once iron enters the blood stream it binds to the transport protein transferrin (Tf). Iron loading of Tf may be facilitated by gastrins in mice exposed to hypoxia (10% oxygen for 10 days), when circulating gastrin levels are increased (35). Tf expression is also regulated in an oxygen-dependent manner by HIF-1 (64). Unexpectedly, macrophage-specific deletion of HIF-1 and HIF-2 did not affect iron recycling. While HIFs are critical regulators of DMT-1 and Fpn in duodenal enterocytes (see above) and of heme oxygenase-1 in some cell types (36), it is not essential for the regulation of the same genes in macrophages during iron recycling (44). Obviously, HIF-mediated transcriptional control of iron genes seems to be a more general principle and may explain the hypoxia-controlled expression of transferrin receptor 1 (39) or ceruloplasmin (50), among others.

In Hypoxia, Iron controls Epo that Controls Erythropoiesis that Controls Heparin that Controls Ferroportin that Controls Iron Levels

The IRE/IRP system that controls cellular iron metabolism is tightly connected to the HIF-mediated hypoxic response. But what occurs at the systemic level when a mountaineer is gaining altitude? The key to systemic iron homeostasis is the amount of iron-bound transferrin in the circulating blood that supplies iron to the expanding mass of erythrocytes, defined as the erythron (31). A physiological concentration of ferric iron-bound Tf that is usually specified in percentage (normal values being between 15–45% Tf saturation) is maintained by the hepcidin/ferroportin regulatory system (31). The small liver-derived peptide hormone hepcidin (25 amino acids) controls dietary iron uptake as well as iron release from iron-recycling macrophages by binding to its target receptor, the iron exporter ferroportin (Fpn), and subsequently triggering Fpn’s internalization and degradation (51). In other words, hepcidin leads to cellular retention of iron in enterocytes and macrophages and therefore to reduced levels of circulating iron. The hepcidin level itself is controlled by systemic iron availability, inflammatory cues, the erythropoietic drive, and hypoxia (31). As for mountaineers ascending to high altitude, several studies have shown that hepcidin serum levels decrease, thereby enhancing Fpn-mediated iron uptake (see, for example, Refs. 3, 60, and 82). The underlying mechanisms have been under extensive debate in recent years, but it became evident from many studies (some performed in mountaineers) that Epo exerts an indirect effect on hepcidin expression (5, 9, 23, 38, 62). We now understand that an intact bone marrow is required for hepcidin suppression in response to hypoxia and that ongoing erythropoiesis is the corresponding driving force.

How can erythroid expansion forward signals to the liver to control hepcidin synthesis? The answer may be through one or most probably several soluble factors that are secreted from erythroblasts. Epo binds to its receptor present on erythroid progenitor cells (see below and Fig. 2) and drives maturation and proliferation of red blood cells via the Jak/Stat signaling pathway (88). In addition, the team of E. Nemeth and T. Ganz (33) recently discovered that once it reaches the bone marrow, Epo triggers expression of erythroferrone (ErFe), a protein that subsequently is secreted by (pro)erythroblasts to ultimately suppress expression of hepcidin in the mouse liver. The Epo receptor is expressed from burst-forming unit-erythroid (BFU-E) cells to erythroblasts, but only colony-forming unit-erythroid (CFU-E) cells and proerythroblasts are Epo responsive, at least in terms of erythropoiesis (91–93). Elevated ErFe mRNA levels have been found in proerythroblasts and most abundantly in basophilic, polychromatic, and orthochromatic erythroblasts (33). Note that although erythroblasts still harbor the Epo receptor (Fig. 2) it does not further regulate erythropoiesis (reviewed by Ref. 14) but upon binding Epo might induce ErFe expression.

Another important observation is that the Epo-driven maturation from CFU-E to erythroblasts takes (at least in vitro) about 7 days to occur. Few additional days are then required for final maturation to adult erythrocytes (Fig. 2). This delay between hypoxia-induced Epo gene expression and increased number of circulating red blood cells is mirrored in the ascending mountaineer. While serum Epo concentrations reach maximal values 19–39 h following ascent to 4,359 m of altitude, hematocrit and hemoglobin levels only showed a tendency to increase during 10 days of stay at that altitude (1). Very similar observations in the kinetics of Epo and hematocrit were made in mountaineers staying at 4,500 m for 7 wk (48). Iron-bound Tf is delivered to the erythroblast via transferrin receptor 1 for hemoglobin synthesis, and as a consequence serum iron levels decrease (Fig. 2). Indeed, a reduction in iron levels is observed for at least up to 4 days at high altitude, indicating that in this acute hypoxic condition dietary iron absorption by enterocytes and release by macrophages cannot match the increased requirements for erythropoiesis. The magnitude and time course of changes differed between studies likely because of different speeds of ascent and level of high altitude (3, 28, 42, 60, 73).

Most studies do not report a change in ferritin levels during a 2–4 day stay at high altitude, suggesting that tissue iron stores are not detectably depleted within this short time period at high altitude.

Hepcidin production is suppressed at high altitude. Low hepcidin serum levels enhance iron export from macrophages and duodenal enterocytes, and as a consequence iron will be supplied to the blood stream to satisfy the erythropoietic demand for this metal. Kautz and coworkers (33) showed increased ErFe mRNA expression in (pro)erythroblasts of mice already 4 h after phlebotomy or Epo injection (8,000 IU/kg body wt) that preceded hepcidin suppression. Of note, ErFe expression kinetics appear to be Epo dose dependent, as another group detected elevated ErFe mRNA levels in liver and spleen only 4 days after injecting about one-fifth of the Epo concentration...
used in the original study (23). Using a transgenic mouse line that expresses Epo receptor exclusively in hematopoietic tissue (79), the latter group confirmed that Epo’s impact on hepcidin expression is indirect by showing that the presence of the Epo receptor on liver cells is not required for hepcidin’s upregulation by hypoxia. Moreover, homozygous ErFe-deficient mice failed to suppress hepcidin expression after phlebotomy or Epo supply and showed delayed recovery from anemia compared with wild-type controls (33). Interestingly, apart from the bone marrow, ErFe is expressed in multiple mouse tissues, including testis, intestine, and skeletal muscle. Thus it will be interesting to investigate whether ErFe expression in these organs, especially in the exercising muscle, ultimately contributes to the regulation of iron homeostasis (see below). So far, ErFe’s impact on hepcidin expression upon elevated serum Epo levels has been demonstrated only in mice, but it is expected that ascents to high altitude would also induce this erythroid regulator in people. To this end, a working ELISA to detect ErFe in human serum should be established soon.

Most probably ErFe is not the only circulating factor repressing hepcidin expression under hypoxic conditions. Previous studies indicate that in healthy volunteers, during a 7-day sojourn to altitude (4,340 m above sea level) Epo and hepcidin levels did not correlate (82). In another study 23 healthy volunteers were subjected to exercise under hypoxic conditions, equivalent to an altitude of 5,600 m (76). Six hours after exercise performance an elevated concentration of platelet-derived growth factor (PDGF)-BB was observed, showing a significant correlation with hepcidin levels in these individuals. Importantly, PDGF-BB was also increased in mountaineers ascending to 4,550 m within 2 days (3). A causal relationship between PDGF-BB levels and hepcidin expression could be demonstrated in mice injected with PDGF-BB in which hepcidin was suppressed and circulating iron levels increased (76). Additional erythroid factors that may down regulate hepcidin expression in response to an expanding erythron under hypoxic conditions have been discovered [e.g., growth differentiation factor 15 (GDF15)] when studying diseases of ineffective erythropoiesis, such as thalassemia (83). While GDF15 levels are increased in mountaineers at high altitude, they do not seem to correlate with hepcidin levels (82). Furthermore, analysis of GDF15-deficient mice demonstrates that GDF15 is not essential for the regulation of systemic iron homeostasis in response to phlebotomy (11). In summary, apart from ErFe, PDGF-BB, and GDF15 there may be more hepcidin-regulating factors to be detected in the serum of individuals acutely exposed to hypoxia.

Iron Regulation in the Exercising Mountaineer

The muscles of a mountaineer are exercising when ascending. Of note, skeletal muscles harbor about one-eighth of the body’s iron, mainly in myoglobin and cytochromes. How does exercise influence iron regulation? As mentioned above, ErFe, the newly identified repressor of hepcidin, is expressed in the skeletal muscle, too. In fact, ErFe is identical to the recently discovered muscular factor termed myonectin/CTPR15 that connects skeletal muscle activity to systemic lipid homeostasis in liver and adipose tissue (68). There are conflicting data as to when and how exercise induces ErFe gene expression in the skeletal muscle. In the original paper, Seldin and coworkers (68) report that wild-type mice given access to a running wheel for 2 wk exhibited higher myonectin/ErFe mRNA levels in the muscle as well as higher protein levels in blood. In contrast, others observed that serum myonectin/ErFe concentration dropped in 28 women after a 10-wk period of aerobic exercise.

Fig. 2. Erythropoiesis requires Epo and iron. Erythropoiesis occurs in the bone marrow and describes the process by which erythroid progenitors proliferate and differentiate into reticulocytes and erythrocytes. Two distinct erythroid progenitors, the early-stage burst-forming unit-erythroid (BFU-E) and the later stage colony-forming unit-erythroid (CFU-E) progenitor have been defined. The so-called proerythroblast is the earliest morphologically recognizable erythroblast that undergoes mitosis to produce basophilic, polychromatic, and orthochromatic erythroblasts. The latter expel their nuclei to become reticulocytes. The phase in which Epo stimulates this differentiation process is indicated. Additionally Epo’s binding to proerythroblasts and mainly to erythroblasts activates the expression of ErFe, a critical suppressor of hepcidin expression in the liver. Ultimately, Tf-bound iron is taken up via the transferrin receptor-1 (CD71), which is highly expressed during all maturation stages but is absent in the mature red blood cell (52).

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performance at submaximal oxygen consumption (37). To make it even more confusing, a very recent study on aerobically trained rats reported that after 9 wk of exercise, myoglobin mRNA was downregulated in the skeletal muscle while protein level increased (59). Thus the impact of exercise on ErFe serum levels has yet to be sorted out. If it turns out that muscular ErFe synthesis does not interfere with serum ErFe levels, then the latter could possibly be used as a new biomarker to detect Epo abuse (G. Cairo, personal communication).

How else is iron homeostasis challenged in our exercising mountaineer during ascent? Both exposure to high altitude (reviewed in Ref. 15) and exercise performance (13) elevate inflammation markers, especially that of interleukin-6 (IL-6) that is well known to activate hepcidin expression (53–57, 86). Inflammation thus counteracts the hypoxia-induced hepcidin decrease that may cause lower serum iron levels than expected upon exposure to oxygen depletion. Low-altitude conditions may ultimately reduce physical performance and, in addition, may also disturb neuronal function and immune responses (9). This is caused by decreased oxygen transport to the exercising muscle and by deficits of nonheme iron associated enzymes, respectively.

**Stomach and Intestine at High Altitude**

Finally the mountaineer reaches the Italian study hut Capanna Regina Margherita at 4,559 m above sea level, a famous place for high-altitude research, and stays there for 5 days. What happens to iron uptake? First, one has to note that over 50% of mountaineers suffer from acute mountain sickness (AMS) at altitudes over 4,000 m and that nausea is a classical AMS symptom (65, 89). Thus general appetite will be reduced in relation to the AMS severity, but interestingly, gastrointestinal satiation hormones are not involved in appetite loss (2). Gastrointestinal changes also occur at high altitude, such as general downregulation of duodenal solute carrier (SCL) transporters (90). A study performed on Tibetan railroad construction workers showed a 0.5% incidence of life-threatening gastrointestinal bleeding due to ulcer formation (94). In keeping with this, another recent study revealed that patients suffering from inflammatory bowel disease risk exacerbation of their symptoms upon high-altitude journeys or even commercial flights (85).

Unexpected was the recent observation that 14 out of 23 (61%) healthy mountaineers staying their fourth night at 4,559 m displayed peptic mucosal lesions such as erosions, ulcers, and hemorrhagic gastritis/duodenitis (21). It is of interest to learn whether these gastrointestinal lesions disappear after longer acclimation periods to high altitude and whether highlanders such as Tibetans or Quechuas are well adapted and thus unaffected. Nevertheless, in acutely acclimatizing mountaineers all these effects will lead to iron loss. Consequently iron uptake has to be upregulated, but how? There is convincing evidence that relevant mechanisms include an increase of dietary iron absorption. By analyzing blood samples and duodenal biopsies obtained from 25 healthy mountaineers upon reaching the Capanna Regina Margherita, a Zurich team reported a rapid decline in serum iron and ferritin levels, the latter being indicative for iron mobilization stored in various tissues. As expected, the decline in iron and ferritin levels was paralleled by an up to 10-fold elevation of duodenal DMT-1 and Fpn mRNA levels (28). On the other hand, serum Epo levels peaked at the second day spent in the hut, while hepcidin levels were markedly reduced. It is tempting to speculate that the Epo → hepcidin signal is linked by ErFe, which unfortunately cannot be measured (yet). Most probably, these responses to hypoxic exposure allow elevated dietary iron uptake and the release of iron stores ultimately covering the elevated iron demand at high altitude. However, within short study periods (up to 4 days), serum iron levels remain low (see above).

**Iron’s Impact on Pulmonary Hypertension and HAPE**

Reduced oxygen availability as occurring at high altitude leads to pulmonary arterial hypertension because of pulmonary vasoconstriction (63). Under chronic hypoxic conditions the latter represents the consequence of pulmonary vascular remodeling due to endothelial cell proliferation in the lung, accompanied by smooth muscle cell hypertrophy and perivascular inflammation that together contribute to increased pulmonary arterial systolic pressure (PASP). Interestingly, during acute exposure to high altitude, the hypoxically-induced elevation in PASP is blunted upon serum iron infusion prior to hypoxic exposure. This observation indicates that iron-dependent processes play a crucial role in the development of hypoxic pulmonary vasoconstriction (71, 81). Accordingly, iron chelation by infusion of desferoxamine aggravated hypoxic pulmonary vasoconstriction, suggesting that iron deficiency has a hypoxia-mimetic effect and does not simply augment the signal generated by hypoxia (6, 71). In line with these observations, irp1-deficient mice develop pulmonary hypertension that was exacerbated by a low-iron diet (27). In pulmonary endothelial cells, irp1 deletion enhanced expression of the α-subunit of HIF-2 that in turn markedly induced endothelin-1 expression. It seems plausible that the latter vasoconstrictor contributes to the development of pulmonary hypertension observed in homozygous deficient irp1 mice.

In a small percentage of otherwise healthy mountaineers, elevation of PASP is exaggerated at high altitude (for review see Ref. 7), and subsequently increased hydrostatic pressure in pulmonary capillaries can be observed (41). High pressure in turn causes fluid leakage (most probably through the interaction of HIF-1 and aquaporin-1) (E. Swenson, personal communication, and Haider and Gassmann, unpublished observations), plasma proteins, and even erythrocytes into the alveolar space (80). This pathological state that can affect mountaineers at high altitude is termed high-altitude pulmonary edema (HAPE) and can be prevented by decreasing PASP (8, 40). Because low iron availability enhances the pulmonary vascular tone in hypoxia (see above), a recent study postulated that low serum iron levels at sea level or at high altitude may contribute to the exaggerated hypoxic pulmonary arterial hypertension and HAPE (3). However, variation of serum iron levels within the normal clinical range did not correlate with an exaggerated hypoxic pulmonary vasoconstriction or the development of HAPE. Of note, the individuals that developed HAPE were unable to reduce hepcidin levels at high altitude and showed misregulation of hepcidin activators, such as IL-6 or PDGF-BB. We speculate that the resulting elevated hepcidin serum level may contribute to the development of iron deficiency over
time, although this was not seen during the short observation period of the mentioned study (3).

Conclusions

Regulation of cellular and systemic iron and oxygen homeostasis are so closely connected at the molecular level that it is difficult to discern what regulates what. Are the PHDs iron sensors that require oxygen or oxygen sensors that require iron to ultimately control stabilization of the α-subunit of the transcription factor HIF-2? Notably, Frise and Robbins (20) formulates a similar question in his accompanying review article. In addition, HIF-2 orchestrates the expression of genes that maintain iron homeostasis but likewise controls adaptation to chronic hypoxic exposure. Surprisingly, it is not only low iron levels that predominately control expression of the iron hormone hepcidin, but rather the oxygen-responsive blood hormone Epo. Knowledge about this tight relationship between hormone hepcidin, but rather the oxygen-responsive blood hormone Epo. Knowledge about this tight relationship between hormone hepcidin and oxygen sensing with erythropoiesis and iron absorption. The IRP1-HIF-2

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