

### Response of skeletal muscle mitochondria to hypoxia

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This review explores the current concepts relating the structural and functional modifications of skeletal muscle mitochondria to the molecular mechanisms activated when organisms are exposed to a hypoxic environment. In contrast to earlier assumptions it is now established that permanent or long-term exposure to severe environmental hypoxia decreases the mitochondrial content of muscle fibres. Oxidative muscle metabolism is shifted towards a higher reliance on carbohydrates as a fuel, and intramyocellular lipid substrate stores are reduced. Moreover, in muscle cells of mountaineers returning from the Himalayas, we find accumulations of lipofuscin, believed to be a mitochondrial degradation product. Low mitochondrial contents are also observed in high-altitude natives such as Sherpas. In these subjects high-altitude performance seems to be improved by better coupling between ATP demand and supply pathways as well as better metabolite homeostasis. The hypoxia-inducible factor 1 (HIF-1) has been identified as a master regulator for the expression of genes involved in the hypoxia response, such as genes coding for glucose transporters, glycolytic enzymes and vascular endothelial growth factor (VEGF). HIF-1 achieves this by binding to hypoxia response elements in the promoter regions of these genes, whereby the increase of HIF-1 in hypoxia is the consequence of a reduced degradation of its dominant subunit HIF-1 $\alpha$ . A further mechanism that seems implicated in the hypoxia response of muscle mitochondria is related to the formation of reactive oxygen species (ROS) in mitochondria during oxidative phosphorylation. How exactly ROS interfere with HIF-1 $\alpha$  as well as MAP kinase and other signalling pathways is debated. The current evidence suggests that mitochondria themselves could be important players in oxygen sensing. *Experimental Physiology* (2003) **88.1**, 109–119.

The series of reviews in this issue considers mitochondria, their structure and function as well as the molecular aspects of mitochondrial plasticity. The focus on mitochondria neglects the fact that for the dominant function of mitochondria, aerobic ATP re-synthesis, the mitochondrial organelle cannot function independently. It is embedded, at least in animal phyla, in a systems physiological context. Mitochondria house the final biochemical steps in the production of reducing equivalents that react at the terminal oxidases of the respiratory chain with molecular oxygen provided by the respiratory system from the environment. In mitochondria, O<sub>2</sub> finally disappears and oxygen partial pressure goes to zero (Fig. 1). Mitochondria thus are an effective oxygen sink and this allows organisms to use all of the available oxygen partial pressure of the actual environment to drive the respiratory cascade from lungs through circulation to the mitochondria (Taylor & Weibel, 1981). Considering, as is the focus of this review, the response of skeletal muscle mitochondria to a lowered environmental oxygen partial pressure ( $P_{i,O_2}$ ), would therefore require an understanding

of the exact interdependence of environmental oxygen availability and mitochondrial oxidative phosphorylation in the entire animal. This understanding is currently incomplete. What is known is that isolated mitochondria do not need more than a few torr of O<sub>2</sub> partial pressure for full function (Gayeski & Honig, 1997). Similarly, it is generally acknowledged that assessing mitochondrial function in hypoxia in cultured cells requires the lowering of the oxygen pressure at the cell surface to values between 0.5 and 5 Torr, values completely incompatible with the survival of all but a few highly specialized species (Boutilier & St Pierre, 2002).

In the context of this review we mostly neglect the hypoxic response of the microvasculature as well as the cardiovascular and pulmonary system, which all conspire to maintain mitochondrial function under hypoxic conditions (Hochachka, 1998). We recognize that the designation of the type of hypoxia intervention (duration and/or intervals) has not been handled uniformly in the literature, including work published by our group. For the sake of consistency in discussing the effect of hypoxia on

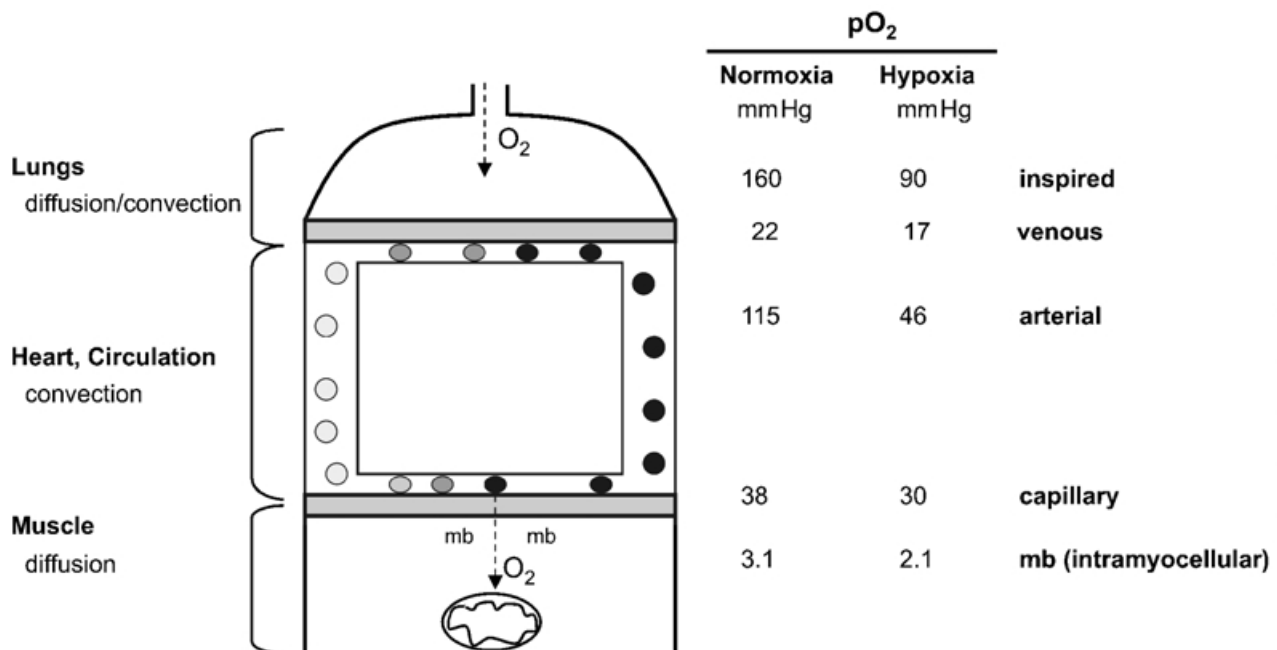
Dedicated to the memory of Peter W. Hochachka (1936–2002).

mitochondria in organisms exposed to hypoxia we propose the following nomenclature: 'native' to hypoxia designates organisms that have been exposed to hypoxia for generations and may genetically be equipped to perform at altitude, as has been proposed for Sherpas or Quechus (Kayser *et al.* 1996b; Hochachka, 1998). We use 'permanent' hypoxia for conditions under which organisms live under hypoxic conditions from birth to death and may only for brief periods of time and as an exception be exposed to normoxia (example: residents of LaPaz; Favier *et al.* 1995). With 'long-term' hypoxia we designate conditions under which organisms or subjects are brought into hypoxia and stay there for weeks or months, thus giving rise to a suite of acclimatization processes (i.e. mountaineering expedition to the Himalayas; Hoppeler *et al.* 1990a). With 'short-term' hypoxia we refer to continuous bouts of hypoxia exposure lasting from minutes to hours (such as during a physical exercise bout), whereby during most of the day subjects are subjected to normoxic conditions. Short-term hypoxia interventions are currently used in athletics to elicit specific hypoxia responses of the organism such as an increase in circulating erythropoietin and red cell mass (Levine & Stray-Gundersen, 1997; typical exposure time, several hours during sleep) or skeletal muscle adaptations (Vogt *et al.* 2001); typical exposure time, 30 min to 2 h during exercise). With the term 'intermittent' hypoxia we designate experimental protocols whereby short periods of hypoxia (minutes) are interspersed with similarly short periods of normoxia. The physiological effects of intermittent hypoxia interventions have not yet been well

defined. In particular, these types of interventions have not to our knowledge been analysed with regard to changes of the mitochondrial compartment. This literature will thus not be reviewed here (see Serebrovskaya, 2002). We also recognize that hypoxaemia (combined with hypercapnia) in humans often results from lung disease such as chronic obstructive pulmonary disease (COPD). We will not consider this pathological condition and its consequences for muscle mitochondria in this review. It is known, however, that muscle dysfunction is an important consequence of COPD and that muscle oxidative capacity and bioenergetics are disturbed in these patients (Mador & Bozkanat, 2001).

### Early work on hypoxia and skeletal muscle structure

One of the first notions of a change of muscle oxidative capacity with hypoxia is found in Reynafarje's (1962) landmark paper on muscle adaptations in Peruvian miners. He found cytochrome *c* reductase activity (+78%) and myoglobin content (+16%) to be significantly increased in biopsies of sartorius muscle of permanent high-altitude residents as compared to low-landers. This paper, together with data of larger muscle capillary densities in guinea pigs native to the Andes as compared to data from low-land control animals (Valdivia, 1958), had a strong impact on physiologists' thinking for at least two decades. According to the then held beliefs it was assumed that altitude hypoxia stimulates muscle oxidative capacity in order to compensate for the lack of oxygen in the atmosphere. When it became clear towards the end of the sixties, that endurance-type exercise was also a potent



**Figure 1**

The pathway of oxygen. Simplified model of the oxygen transport pathway showing the principal structures and the corresponding partial pressure of oxygen ( $P_{O_2}$ ) in these compartments, during maximal exercise, while breathing normoxic or hypoxic room air. mb, myoglobin. The data are derived from Richardson *et al.* (1995).

stimulator of muscle mitochondrial enzyme activity (Holloszy, 1967), the connection was made that local tissue hypoxia provoked by exercise might be an important signal for mitochondrial proliferation under heavy exercise conditions. The consensus view of the role of hypoxia for the muscle oxidative machinery in mammals was most distinctly formulated by Hochachka in 1982 in a paper entitled 'Metabolic meaning of elevated levels of oxidative enzymes in high-altitude adapted animals: an interpretive hypothesis' (Hochachka *et al.* 1982). Based on the then available evidence he suggested that 'The system is obviously designed to maintain ATP synthesis in the face of changing O<sub>2</sub> availability down to very low O<sub>2</sub> tensions ... in principle by increasing enzyme activities per g of tissue'.

However, with time, the prevailing view of the impact of hypoxia on muscle oxidative capacity did not remain uncontested. Banchero (1987) had reviewed the literature on experimental hypoxia exposure of animals and came to the conclusion that exposing animals to hypoxia alone was not sufficient to induce muscle structural changes but that these would result from combining hypoxia with either cold or exercise. A re-appraisal of Reynafarje's (1962) data from a historical perspective also revealed that in fact the basic tenet of the study, that the increase in mitochondrial enzyme activity was solely due to hypoxia exposure, was probably not true. It was found that Reynafarje, unaware of the effect of exercise on the mitochondrial compartment in muscle, had compared a group of relatively active high-landers to a group of more sedentary low-landers (B. Saltin, personal communication).

### Long-term hypoxia on mountaineering expeditions

When we had the opportunity to analyse muscle samples of mountaineers obtained before and after expeditions to the Himalayas we expected to find mitochondrial and capillary densities to be increased after return from expeditions. The findings turned out to be more complex than that (Hoppeler *et al.* 1990b; Howald *et al.* 1990). The capillary density (i.e. the number of capillaries counted per unit area of muscle fibre) was indeed found to be increased in post-expedition samples. However, this was not due to capillary neo-formation since the capillary to muscle fibre ratio was unchanged. What had occurred over the time of the expedition was a significant loss of muscle fibre cross-sectional area. Hence, the same number of capillaries supplied a smaller tissue space. In addition, we found a decrease of mitochondrial volume density, which led to a total loss of muscle mitochondrial volume of close to 30%. As a consequence we found  $V_{O_{2,max}}$  significantly reduced upon return from the Himalayas (Ferretti *et al.* 1991). Our results were confirmed by studies appearing at about the same time of the analysis of muscle samples obtained from subjects exposed to hypobaric hypoxia in a pressure chamber during a simulation of an ascent to Mt Everest 'Expedition Everest II'. The simulated ascent also led to a loss of muscle volume, did not induce capillary neo-formation and led to a net loss of muscle oxidative capacity (MacDougall *et al.* 1991). The authors of this study questioned whether the tenet of hypoxia as an important

signal for capillary and mitochondrial neo-formation could be maintained in the face of the conflicting data. Our structural analysis produced additional evidence of muscle tissue stress due to long-term exposure to hypoxic conditions. We found the volume fraction of lipofuscin inclusions (Fig. 2) to be significantly increased by over 2-fold after return from the expeditions (Martinelli *et al.* 1990). Lipofuscin is believed to be a degradation product of mitochondria, prevailing under conditions of increased oxygen radical formation (Brunk & Terman, 2002). At the time we were unable to give an explanation for our findings; however, in the light of current evidence of ROS formation in muscle mitochondria (Pearlstein *et al.* 2002), our early structural results on mitochondria degradation after long-term hypoxia exposure appear in a new light (see below).

Recent experiments with rats hint at the molecular changes possibly responsible for the functional adaptations in skeletal muscle to chronic hypoxia. Protein expression of the mitochondria-associated anti-apoptotic gene, Bcl-2, was found to be inversely related to the oxidative characteristics of muscles and markedly induced in rat muscles after 3 weeks of exposure to chronic hypoxia. This adaptation was suggested to represent an anti-apoptotic mechanism allowing protection against the lack of oxygen in oxidative muscles (Riva *et al.* 2001).

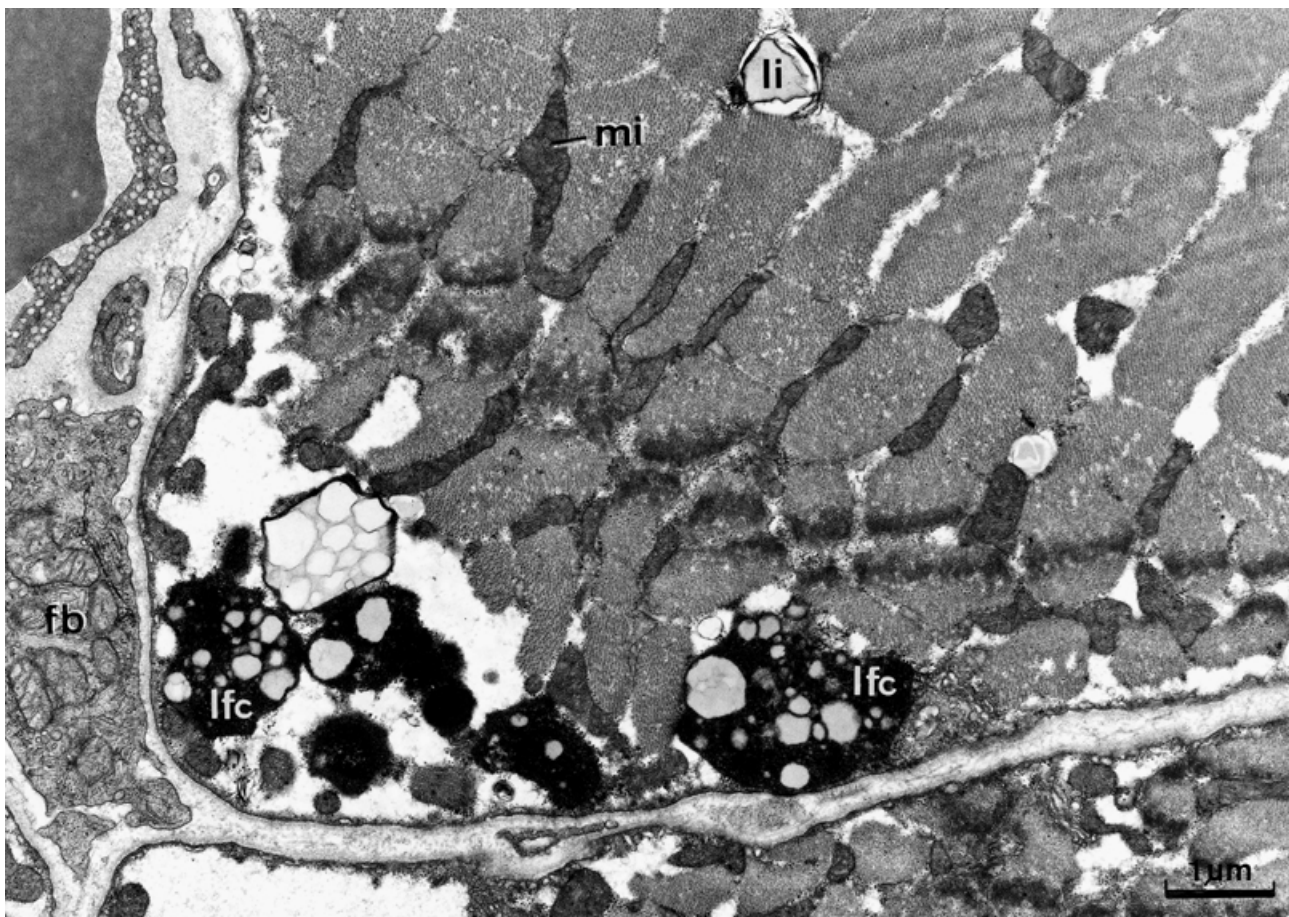
### Permanent hypoxia and muscle structure

One possible explanation for the unexpected finding of a decrease of muscle mitochondrial oxidative capacity with long-term hypoxia exposure could be the extraordinary levels of stress in a pressure chamber experiment as well as the general hardship associated with climbing in the Himalayas for weeks. We therefore set out to analyse muscle tissue of people permanently exposed to hypoxia but living under perfectly normal conditions. We studied the muscle biopsies of 30 students, residents of La Paz (3400–4000 m; Favier *et al.* 1995). We found these subjects to be smaller and lighter than age-matched European low-land controls (Desplanches *et al.* 1996). Interestingly, fibre size (3507 vs. 3513  $\mu\text{m}$ , respectively) was almost identical between these high-landers and low-landers, whereas mitochondrial volume density was significantly lower by 19% in high-landers. Incidentally, capillary supply in high-altitude residents was also decreased by 20%. A corollary finding of this study was that of a decrease of the intramyocellular fat content to less than half of what was observed in low-landers. It could not be established whether this difference was due to differences in dietary habits, i.e. whether these high-land subjects consumed less fat in their diet or whether this is a direct effect of hypoxia on substrate metabolism of muscle cells (Hoppeler *et al.* 1999; Vogt *et al.* 2001). It is well established that high-altitude exposure and acclimatization increases glucose utilization both at rest and during exercise (Brooks *et al.* 1991). This might well be one of the important metabolic consequences of hypoxia-inducible gene expression discussed in more detail below (Fig. 3). Transcription of a large number of genes is induced by hypoxia; among them

many involved in glycolysis as well as in glucose transport across the sarcolemma (Semenza, 1999). We would assume that the increased transcription of genes involved in the metabolic pathways favouring glucose metabolism in muscle cells is responsible for the observed shift towards glucose metabolism in organisms at altitude. The functional advantage of this adaptation would be a larger ATP yield per molecule of oxygen (Wilmore & Costill, 1994). It has recently been shown that protein expression of the enzymes and transporters of lactate, lactate dehydrogenase (LDH) and monocarboxylate transporters (MCT1, 2 and 4), is affected in a tissue-specific manner by long-term exposure to hypobaric hypoxia (McClelland & Brooks, 2002). These findings are broadly compatible with the idea that hypoxia induces mechanisms favouring glucose utilization in muscle cells.

Low mitochondrial contents were also observed when biopsies of Sherpas were analysed. We found comparatively low levels of mitochondrial volume density (3.96%) in these subjects (Kayser & Hoppeler, 1991) believed to be of

a high-altitude lineage resident to the Himalayas for hundreds of generations (Hochachka, 1998). The low level of mitochondrial volume density has also been observed in second generation Sherpas raised at low-altitude and might therefore possibly have a hereditary component (Kayser *et al.* 1996a). Despite the low muscle mitochondrial oxidative capacity, physical performance capacity of native high-land populations at altitude is excellent. A number of physiological mechanisms have been invoked to explain the 'paradoxical' finding of superior aerobic performance in hypoxia despite modest muscle oxidative capacities (Hochachka *et al.* 2002). Hochachka *et al.* (1998) describe systemic mechanisms, among them being a blunted hypoxia ventilatory and pulmonary vasoconstrictor response as well as an up-regulation of erythropoietin expression, which help native high-land populations to excel at altitude. At the level of muscle tissue it is suggested that the control contributions from cellular ATP demand and ATP supply pathways are up-regulated whereas the contribution of control steps in O<sub>2</sub> delivery are down-regulated (Hochachka *et al.* 2002). This leads to an improved



**Figure 2**

Long-term exposure of low-landers to hypoxia causes lipofuscin accumulation in muscle cells. Micrograph of cross-sections of vastus lateralis muscle fibres of a mountaineer after return from an expedition to the Himalayas. Conspicuous accumulation of lipofuscin are evident near the sarcolemma of one fibre (lfc = lipofuscin; mi = mitochondria; li = lipid droplet; fb = fibroblast in interstitial space).

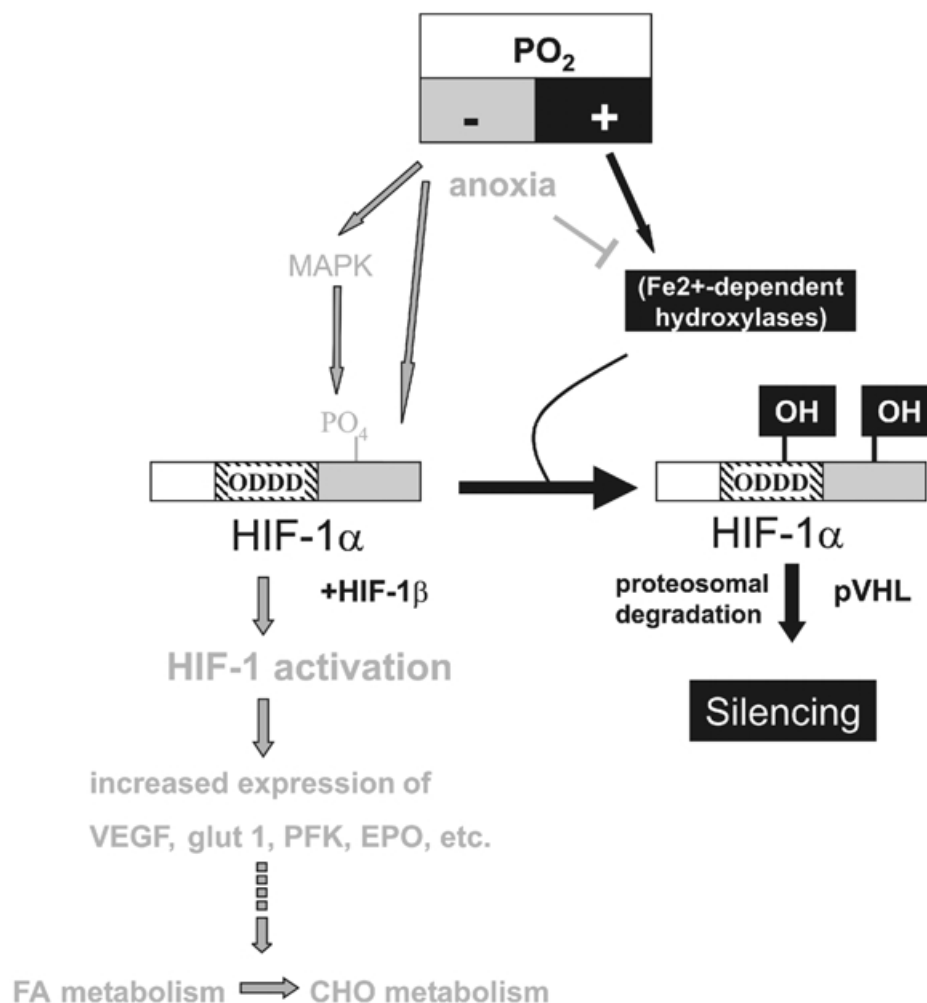
coupling between ATP demand and supply pathways and better metabolite (i.e. lactate, adenylates) homeostasis.

From the results of the studies exploring permanent or long-term hypoxia exposure a clear picture emerges. Muscle mitochondria content is reduced in all subjects spending long time periods at high altitude. Moreover, it looks as if at least low-landers do not tolerate hypoxia very well and show sign of considerable muscle wasting and an accumulation of lipofuscin granules in muscle cells. Highland populations seem to be better adjusted to living at altitude. They also have lower mitochondrial oxidative capacities but they seem to have (genetic?) systemic and

local muscle adaptations allowing them to function better in hypoxia. From this it would appear that there would be very little advantage to preparing low-land athletes for competition by using hypobaric or normobaric hypoxia. How is it then that altitude training has been practised at least occasionally with apparent success?

### Short-term hypoxia exposure

We reasoned that hypoxia might still be an important stimulus related to exercise in muscle tissue. However, the stimulus of hypoxia could be negated under chronic altitude conditions such that hypoxia may negatively



**Figure 3**

HIF-1 $\alpha$ -mediated oxygen sensor and hypoxia-inducible gene expression. Oxygen stabilizes hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) through Fe<sup>2+</sup>-dependent proline hydroxylases and asparaginyl hydroxylase(s) which hydroxylate HIF-1 $\alpha$  within its oxygen-dependent degradation domain (ODDD) and C-terminal portion, respectively. Such modification causes recruitment of the von Hippel-Lindau tumour-suppressor protein (pVHL), which targets HIF-1 $\alpha$  for proteosomal degradation thereby silencing HIF-1 $\alpha$  activity. Conversely, hypoxia causes a stabilization of HIF-1 $\alpha$  and activates the MAPK pathway which enhances the transcriptional activity of HIF-1 $\alpha$  through phosphorylation. Both mechanisms contribute to transcriptional activation of downstream angiogenic factor (VEGF), erythropoietin (EPO), glucose transporters (glut 1 and 3) and glycolytic genes (PFK) via HIF-1 $\alpha$ /HIF-1 $\beta$  dimers (HIF-1). Moreover, lack of oxygen (anoxia) causes HIF-1 $\alpha$  stabilization, possibly by reducing the activity of hydroxylases by depleting their substrate O<sub>2</sub>.

interfere with recovery processes including signalling events, transcription, translation and protein synthesis. We thus decided to explore hypoxia protocols under which hypoxia was present only during the exercise session but not during recovery (e.g. living low–training high). Four groups of initially untrained subjects were set up to train five times a week for a total of 6 weeks on a bicycle ergometer. Two of these groups trained at 560 m (normoxia) and two at simulated 3850 m (hypoxia;  $F_{i,O_2}$  of 13%). For each of the oxotensic groups, one trained at the anaerobic threshold (high intensity) and the other at about 25% below this level (low intensity). Analysis of pre- and post-training biopsies of m. vastus lateralis revealed that total mitochondria increased significantly in all groups; in contrast, subsarcolemmal mitochondria, i.e. those located near capillaries, increased significantly only in those groups training under hypoxic conditions, irrespective of training intensity. Noticeably, the group which trained at high intensity in hypoxia showed the highest increase in total mitochondrial volume density (+59%) and capillary length density was increased significantly in this group only (+17.2%) (Geiser *et al.* 2001; Vogt *et al.* 2001). These results indicate that strenuous training in hypoxia while living near sea-level leads to muscle adaptations which compensate the reduced availability of oxygen by improving the conditions for transportation and utilization of oxygen in exercising muscle.

Several studies performed under the paradigm of ‘living low–training high’ indicate that performance or  $V_{O_{2,max}}$  in hypoxia is improved more after hypoxia than under normoxia training (Terrados *et al.* 1988, 1990; Desplanches *et al.* 1993; Bailey *et al.* 2000; Meeuwsen *et al.* 2001), while some others do not (Emonson *et al.* 1997; Melissa *et al.* 1997; Ventura *et al.* 2002). Despite these discrepancies reported for functional changes, it was shown that the activity of citrate synthase was increased more after training under hypoxic conditions than after the same training under normoxic conditions (Terrados *et al.* 1988; Melissa *et al.* 1997; Green *et al.* 1999). These results support our finding of a hypoxia-induced increase of muscular oxidative capacity and mitochondrial density (Geiser *et al.* 2001). Furthermore, improvements of anaerobic performance were shown by Meeuwsen (Meeuwsen *et al.* 2001) when he trained eight cyclists at a simulated altitude of 2500 m and another group at sea-level, 2 h per day for 10 days. Beside significant increases in  $V_{O_{2,max}}$  and maximal power output, a Wingate test showed increased anaerobic performance parameters only for the hypoxia training group.

Analysis of mRNA expression in the muscle biopsies from the subjects of our hypoxia training study hint at the molecular changes possibly responsible for the functional adaptations in skeletal muscle to hypoxia. The mRNA of hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ), which drives transcription of hypoxia-inducible genes (Semenza, 1999), is increased after training under hypoxic conditions irrespective of training intensity, but not after training in normoxia (Vogt *et al.* 2001). Moreover, we found VEGF,

myoglobin and phosphofructokinase mRNA increased with training at high intensity in hypoxia but not in normoxia. The expression of the latter two genes can be induced by HIF-1 $\alpha$  following its stabilization in hypoxia (see below; Semenza, 1999).

In conclusion, molecular and functional results reveal that a hypoxic stimulus, which is only present during an exercise session, can lead to additional muscular and systemic adaptations as compared to the same training regime in normoxia. For athletes, hypoxia training can be a way to improve performance for competition at altitude and eventually also at sea-level. In practice, athletes can train under simulated hypoxic conditions (Vogt *et al.* 1999) by using a portable oxygen diluting device (Altitrainer 200, SMT, Switzerland) or by going to real altitude or to a pressure chamber for the exercise session only.

As indicated above, the consensus of the studies using short-term hypoxia during exercise sessions seems to be that exercise in hypoxia, followed by recovery in normoxia, produces more intense training effects similar to but not identical with the effects seen in normoxia (Hoppeler & Vogt, 2001). The reason why long-term hypoxia exposure may be damaging for muscle cells has not yet been elucidated and it may well be a multifactorial process. There are indications that protein synthesis is compromised in hypoxia (Gracey *et al.* 2001). However, other factors may play a role as well.

### Mechanisms related to local hypoxia in exercised skeletal muscle

Several independent lines of evidence suggest the occurrence of ‘local hypoxia’, i.e. a drop in intramyocellular oxygen pressure with exercise. Spectroscopic data on myoglobin saturation in frozen dog gracilis muscles 20 years ago demonstrated a fall in intracellular  $P_{O_2}$  between 15 and 30 s after the onset of electrical stimulation (Gayeski *et al.* 1985). This finding of a low muscle oxygen tension during exercise is supported by more recent data using  $^1H$ -nuclear magnetic resonance spectroscopy which demonstrates that myoglobin desaturation occurs within 20 s of onset of exercise in human quadriceps muscle (Richardson *et al.* 1995). Using the same technique, Richardson *et al.* (2001) have recently shown that oxygen saturation in human skeletal muscle, calculated from myoglobin desaturation, is reduced to a plateau with the onset of exercise. This has been taken to indicate that local hypoxic conditions may prevail in muscle even during low intensity exercise in normoxia. This reasoning is based on the assumption that oxygen is homogeneously distributed within and among muscle fibres once it has been extracted from the capillaries across the steep  $O_2$  gradient into the myofibres (Gayeski & Honig, 1986).

This may, however, not be the case since the spatial resolution underlying these conclusions may have been overstated (Wagner, 2000). Mitochondria, the oxygen sink, are more prevalent in oxidative fibres by a factor of at least three, and even within fibres they are clustered in the fibre periphery close to the capillaries (Howald *et al.* 1985).

As a consequence we would assume that the average deoxy-myoglobin signal in  $^1\text{H}$  nuclear magnetic resonance spectroscopy does not account for local hypoxia conditions within individual fibres, but rather reflects the overall oxidative character of recruited fibres. However, it is these local conditions, which are likely to be relevant to the molecular response of muscle fibres (Gayeski & Honig, 1977; Lindstedt & Wells, 1988).

In the same study cited above, Richardson *et al.* (1995) showed that the breathing of hypoxic air (12%  $\text{O}_2$ ) during incremental quadriceps exercise at maximum work rate causes a greater decrease in myoglobin saturation relative to breathing room air (60% vs. 51% myoglobin desaturation) and hence additionally reduces juxta-mitochondrial oxygen pressure (Fig. 1). This indicates that reductions in ambient  $\text{O}_2$  concentration could influence the phenotypic adaptations of skeletal muscle seen with exercise through causing an additional drop in the tissue level of oxygen. Studies done on healthy young men after acute exposure to high altitude (4300 m, barometric pressure = 463 mmHg) increases glucose use during rest and submaximal exercise (Roberts *et al.* 1996). These data support the notion that hypoxia even in the absence of exercise influences muscle  $P_{\text{O}_2}$  and metabolic processes, thereby provoking a shift towards increased use of the glycolytic pathway for energy production.

The transcription factor HIF-1 (hypoxia-inducible factor 1) acts as a master regulator for the expression of genes involved in the hypoxia response of most mammalian cells (Fig. 3; Semenza, 1999). HIF-1 is a heterodimer composed of a dominant  $\alpha$  (HIF-1 $\alpha$ ) and a  $\beta$  subunit (HIF-1 $\beta$ /ARNT; Semenza, 1999). Upon exposure of cell cultures and tissues to hypoxia, HIF-1 $\alpha$  is rapidly stabilized and accumulates in the nucleus (Jewell *et al.* 2001; Stroka *et al.* 2001). Subsequently, the number of HIF-1 $\alpha$ /ARNT (HIF-1) dimers is increased, which in turn induces the expression of a variety of hypoxia-inducible genes including glucose transporters, glycolytic enzymes, angiogenic factor VEGF and erythropoietin through binding to *cis*-acting hypoxia response elements (HREs) in the promoter region (Semenza, 1999; Pages *et al.* 2000). The increase in HIF-1 $\alpha$  in hypoxia (and anoxia) is achieved by interference with proline hydroxylase activity. In normoxia, HIF-1 $\alpha$  is targeted mainly by hydroxylation of proline in its oxygen-dependent degradation domain (ODDD) for destruction via the ubiquitin pathway (Zhu & Bunn, 1999; Jaakkola *et al.* 2001; Wenger & Bauer, 2001; Fig. 3).

The drop in intramyocellular oxygen tension with onset of exercise indicates that HIF-1 $\alpha$  may be stabilized in exercised skeletal muscle, although this needs to be demonstrated. As shown by Neuffer *et al.* (1998), an increased mRNA steady-state level can reflect a regular activation of the specific protein. Therefore, the higher HIF-1 $\alpha$  mRNA concentration in *m. vastus lateralis* of hypoxic vs. normoxic ergometer-trained subjects (Vogt *et al.* 2001) might reflect an increased potential for transient activation of the HIF-1 protein due to the daily hypoxic stimuli in the altitude-trained groups. *In vitro* experiments

show that an activation of HIF-1 leads to an up-regulation of anaerobic enzymes of the glycolytic pathway (Wenger, 2002). The concomitant increase in mRNA of HIF-1 $\alpha$  with mRNA for the glycolytic enzyme phosphofructokinase and anaerobic performance in hypoxia-trained subjects is suggestive of a scenario whereby hypoxia-stabilized HIF-1 $\alpha$  may drive the selective up-regulation of glycolytic enzymes in hypoxia-trained subjects as well as the shift of metabolism towards an increased use of carbohydrate-derived pyruvate as substrate for mitochondria.

### ROS production during hypoxia: are mitochondria oxygen sensors?

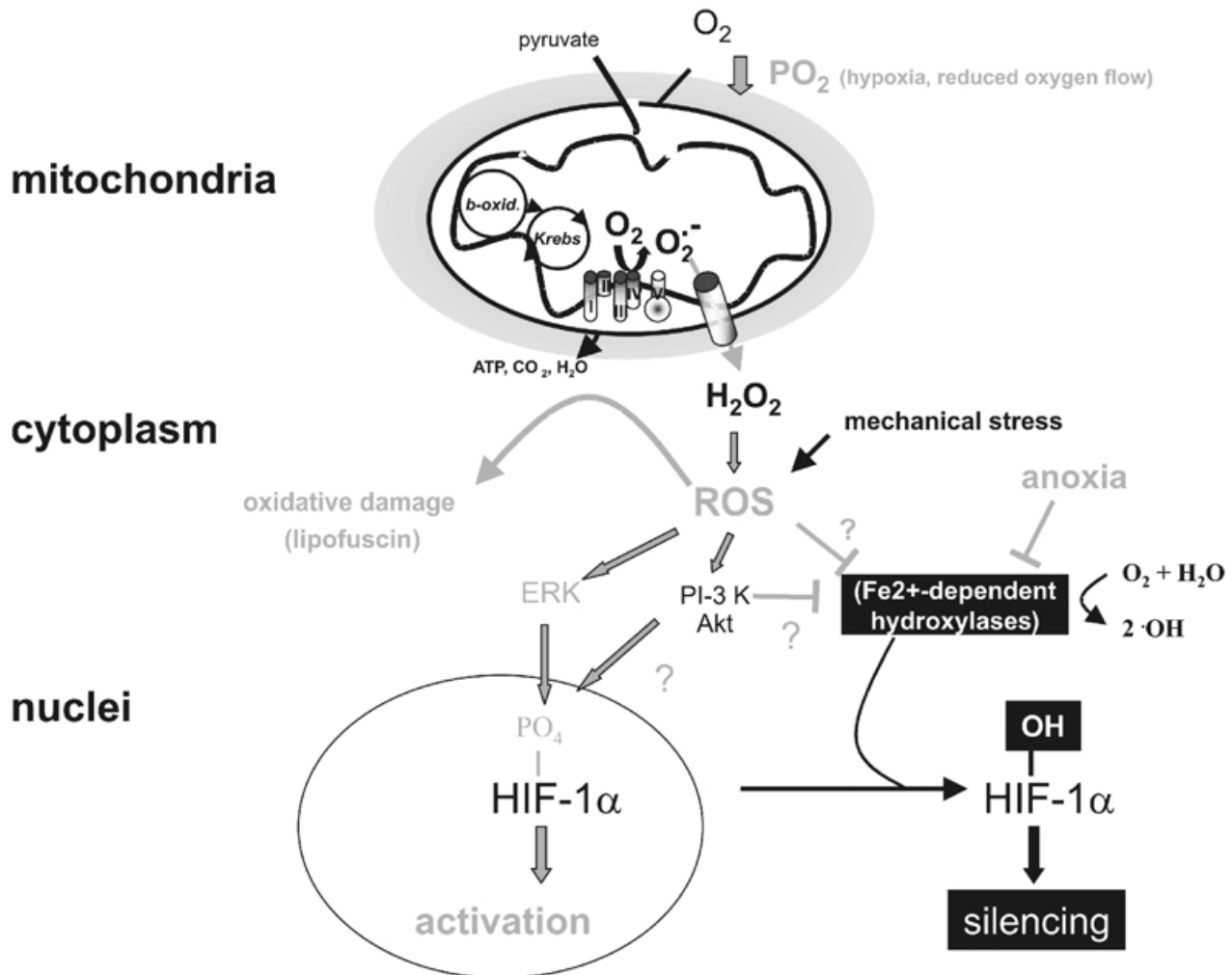
To date there is no clear-cut explanation for the paradoxical finding of increased reactive oxygen species (ROS) production in hypoxia as there is controversy about whether hypoxia, in fact, causes an increase or a fall in ROS production (Archer & Michelakis, 2002). Also the mechanisms by which ROS and cytosolic  $\text{H}_2\text{O}_2$  levels are involved in stabilization/activation and eventual degradation/silencing of HIF-1 $\alpha$  and other redox-sensitive transcription factors are controversially discussed (Chandel *et al.* 2000; Kietzmann *et al.* 2000).

It is reported that ~2% of the  $\text{O}_2$  used by the mitochondrial electron transport chain creates ROS and in particular the superoxide radical, due to its incomplete reduction (see Kietzmann *et al.* 2000; Archer & Michelakis, 2002; reviewed in Abele *et al.* 2002). The superoxide anion ( $\text{O}_2^{\bullet-}$ ) radical is very unstable and is rapidly converted either spontaneously or after its export into the cytoplasm by mitochondrial and cytoplasmic superoxide dismutases, MnSOD and Cu/ZnSOD, to the more stable  $\text{H}_2\text{O}_2$ . Recent findings in hepatoma cells indicate a small (approximately 2.5-fold) and transient increase in ROS after 40–50 min of hypoxia onset (0.5%  $\text{O}_2$ ; Vanden Hoek *et al.* 1998). These and subsequent *in vitro* experiments in the same system point to mitochondria, and to respiratory complex III of the electron transport chain in particular, as the source of ROS production in hypoxia (Chandel *et al.* 2000).

Oxygen radicals (i.e. ROS) are known to damage membranes and oxidize proteins and have been recognized as activating multiple pathways that influence gene expression (Chandel *et al.* 2000; Kietzmann *et al.* 2000). For example, mitochondria and mitochondria-derived ROS are required for the stabilization of HIF-1 $\alpha$  by mitogen-activated protein kinases (MAPK) and by exposure of hepatoma cells to 6 h of hypoxia (1.5%  $\text{O}_2$ ; Chandel *et al.* 2000; Chandel & Schumacker, 2000; Minet *et al.* 2001). This hypoxic stabilization of HIF-1 $\alpha$  in hepatoma cells is blocked by the overexpression of catalase, which catalyses dismutation of  $\text{H}_2\text{O}_2$  into  $\text{H}_2\text{O}$  and  $\text{O}_2$ , and is an inhibitor of the mitochondria superoxide anion channel. By contrast, the addition of  $\text{H}_2\text{O}_2$  in the absence of a hypoxic stimulus causes stabilization of HIF-1 $\alpha$  (Chandel *et al.* 2000). Anoxia, on the other hand, may stabilize HIF-1 $\alpha$  essentially through depletion of the co-substrate dioxygen of prolyl-hydroxylases (Jaakkola *et al.* 2001). This indicates that increases in cytosolic  $\text{H}_2\text{O}_2$  in

consequence of hypoxia-driven ROS production may cause HIF-1 $\alpha$  accumulation in hypoxia through reduction of its hydroxylation (Chandel *et al.* 2000; Figs 3 and 4). Moreover, ROS-activated signalling events involving MAPK and PI-3 K/Akt have been implied in the modulation of the transcriptional activity of HIF-1 $\alpha$  (Richard *et al.* 1999; Minet *et al.* 2001). Last but not least, ROS production potentially could explain the accumulation of the by-products of radical metabolism, such as the breakdown product of mitochondria, lipofuscin (Brunk & Terman, 2002), in low-landers after having spent weeks at high altitude (Martinelli *et al.* 1990).

The much faster stabilization of HIF-1 $\alpha$  in HELA cells (within 2 min of exposure to 0.5% O<sub>2</sub>; Jewell *et al.* 2001) than the appearance of ROS production in hepatoma cells (40 min at 0.5% O<sub>2</sub>; (VandenHoek *et al.* 1998) indicates that mitochondrial ROS production may not necessarily be involved in the instantaneous stabilization of HIF-1 $\alpha$  observed in hypoxia. These differences point to the need for a critical re-interpretation of the data in terms of the differences in the selectivity of the applied detection systems towards the multiple reactive oxygen species (O<sub>2</sub><sup>•-</sup>, OH, NO, ONOO<sup>•-</sup>), the time course and the cell type(s) studied, in order to understand the mitochondrial ROS



**Figure 4**

Targets of mitochondrial ROS production. Incomplete reduction of  $O_2$  during normal metabolic conversion of pyruvate in mitochondria gives rise to a low level of superoxide anion ( $O_2^{\bullet-}$ ). Exposure of cells to hypoxia increases the aberrant production of  $O_2^{\bullet-}$  at the mitochondrial electron chain complex III. Catalysed or spontaneous dismutation of the unstable superanion and export via anion channels enhances the concentration of cytosolic  $H_2O_2$  and other reactive oxygen species (ROS). Increased ROS may increase the level of oxidatively damaged lipids (lipofuscin) or activate downstream redox-sensitive signal transduction events. For example ROS stabilize HIF-1 $\alpha$  possibly by interfering with the activity of  $Fe^{2+}$ -dependent proline hydroxylases. ROS may also activate the MAPK or PI-3K/Akt pathway, which enhance the transcriptional activity of HIF-1 $\alpha$  through its phosphorylation. Lastly, mechanical stress may also increase ROS production which may influence HIF-1 $\alpha$  activation.

response in hypoxia. However, mitochondrial ROS production is recognized as being subject to gradients in oxygen tension. In this regard, it may be plausible that any imbalance in mitochondrial redox potential would at some point result in the production of radicals at any complex of the mitochondrial electron chain (X. Leverve, personal communication in 2001). Such a scenario would in particular be expected in hypoxia, when the respiratory chain components are in a maximally reduced redox state, due to a lower rate of reduction of oxygen at complex IV as a result of oxygen deprivation (reviewed in Abele *et al.* 2002).

Clearly there is evidence pointing to a role of mitochondria as oxygen sensors by modulation of ROS production. These processes could then be linked to downstream modification of transcription factors and lipidic membrane components ultimately underlying the structural–functional alterations seen with exposure of skeletal muscle to hypoxia. Recently, evidence for increased oxidant production in rat skeletal muscle during prolonged exercise has been provided, with both the mitochondrial respiratory chain and the NADPH oxidase as potential sources for oxidants (Bejma & Ji, 1999). This argues for ROS production in skeletal muscle due to local tissue hypoxia. Future studies are now needed to test the relevance of mitochondrial ROS production in consequence of local hypoxia produced by increased muscle activity in intact skeletal muscle.

### Conclusions

On a systemic level, hypoxia influences mitochondria content and hence tissue oxidative capacity in skeletal muscle cells. On a molecular level, mitochondria are involved in hypoxia signalling, in which a number of other signalling pathways seem to cooperate (Hoppeler & Flück, 2003). However, we have not yet reached agreement on the mechanisms and their interplay responsible for the structural and functional modifications of skeletal muscle mitochondria in hypoxia.

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