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HIF-1-driven skeletal muscle adaptations to chronic hypoxia: molecular insights into muscle physiology

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HIF-1-driven skeletal muscle adaptations to chronic hypoxia: molecular insights into muscle physiology

F. B. Favier[1,2] · F. A. Britto[1,2] · D. G. Freyssenet[3] · X. A. Bigard[4] · H. Benoit[5,6]

Abstract Skeletal muscle is a metabolically active tissue and the major body protein reservoir. Drop in ambient oxygen pressure likely results in a decrease in muscle cells oxygenation, reactive oxygen species (ROS) overproduction and stabilization of the oxygen-sensitive hypoxia-inducible factor (HIF)-1α. However, skeletal muscle seems to be quite resistant to hypoxia compared to other organs, probably because it is accustomed to hypoxic episodes during physical exercise. Few studies have observed HIF-1α accumulation in skeletal muscle during ambient hypoxia probably because of its transient stabilization. Nevertheless, skeletal muscle presents adaptations to hypoxia that fit with HIF-1 activation, although the exact contribution of HIF-2, I kappa B kinase and activating transcription factors, all potentially activated by hypoxia, needs to be determined. Metabolic alterations result in the inhibition of fatty acid oxidation, while activation of anaerobic glycolysis is less evident. Hypoxia causes mitochondrial remodeling and enhanced mitophagy that ultimately lead to a decrease in ROS production, and this acclimatization in turn contributes to HIF-1α destabilization. Likewise, hypoxia has structural consequences with muscle fiber atrophy due to mTOR-dependent inhibition of protein synthesis and transient activation of proteolysis. The decrease in muscle fiber area improves oxygen diffusion into muscle cells, while inhibition of protein synthesis, an ATP-consuming process, and reduction in muscle mass decreases energy demand. Amino acids released from muscle cells may also have protective and metabolic effects. Collectively, these results demonstrate that skeletal muscle copes with the energetic challenge imposed by O_2 rarefaction via metabolic optimization.

Keywords Altitude · Atrophy · Hypoxia inducible factor · Metabolism · Mitochondria · Oxidative stress

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Introduction

Skeletal muscle is the most voluminous tissue of the body as it represents almost 40 % of body weight and it accounts for a significant part (20–30 %) of basal metabolic rate [1]. Skeletal muscle is a heterogeneous tissue composed of fibers that have distinct contractile and metabolic properties. Type I fibers (also named slow oxidative) are mitochondria-rich cells with high myoglobin content and capillary density responsible for their red colour. Type IIX and IIB fibers (fast glycolytic) have generally larger cross-section area with higher glycolytic machinery and are less perfused, while type IIA (fast oxidative) exhibit an intermediary profile. More than 90 % of the energy produced by muscle cells comes from the aerobic pathway. A reduction in O_2 availability would therefore challenge skeletal muscle homeostasis. This situation will be exacerbated in case of physical exercise, as it will enhance ATP demand.

Reduced O_2 pressure (hypobaric hypoxia) and sustained exercise are experienced by alpinists and mountaineers, and skeletal muscle adaptations in these subjects have been studied for several decades. Pioneering works evidenced the negative effect of hypoxia exposure on skeletal muscle mass. However, hypoxia is not only restricted to mountaineers or high altitude residents, and could result from pathologies such as anemia, vascular abnormalities, heart failure, chronic obstructive pulmonary disease or sleep apnea. Since reduced muscle mass and/or altered metabolism may be associated with enhanced fatigability, sedentary lifestyle and ultimately increased risk of mortality, the fine understanding of molecular responses driven by skeletal muscle hypoxia is of particular interest for athletes, active people, as well as patients. This review attempts to depict the molecular mechanisms underlying skeletal muscle adaptations to chronic hypoxia (i.e. several days) in healthy subjects.

Defining hypoxia and O_2 sensing pathway

Intramuscular O_2 pressure

The first question when dealing with hypoxia is to define what hypoxia means for the cells. Hypoxia literally means under/below oxygen that we could translate by "less oxygen" or even under oxygenation compared to physiological level. Hypoxic environment can be experimentally reproduced by decreasing the proportion of O_2 available in ambient air (normobaric hypoxia), therefore reducing the inspired O_2 fraction (FIO_2) from the normal value of 20.9 %. Another way is to decrease the barometric pressure leading to the reduction in O_2 availability while its proportion into the inspired air remains constant (hypobaric hypoxia). A correspondence between the common altitude levels used in publications, the FIO_2, the ambient and inspired O_2 pressure is presented in Table 1. The definition of hypoxia raises the issue of a threshold in O_2 pressure (PO_2) that will place the cell in hypoxic condition. Data obtained from magnetic resonance spectroscopy experiments (human) [2, 3], via optical method (rat) [4], surface electrode (dog) [5] or microcatheter (human) [6] lead to resting intramuscular PO_2 of $\approx$27 mmHg in normoxic conditions. Few data exist on intramuscular PO_2 during hypoxia: Johnson et al. reported a value of $\approx$10 mmHg after 1 min of breathing 7 % O_2 (equivalent to 8300 m) in rat cremaster muscle [4], while Richardson's group found more than double (23 mmHg) in humans with an inspired O_2 fraction of 10 % ($\approx$5800 m) [2]. During exercise of even slight intensity (unweighted knee extension at 30 % of maximal leg O_2 uptake), PO_2 rapidly drop to $\approx$5 mmHg [3]. There are only subtle changes in intramuscular PO_2 between very light and heavy exercise as PO_2 decreases to 3 mmHg at 50 % of maximal leg O_2 uptake and remains constant until maximal work rate. During hypoxic trial ($FIO_2 = 12$ %), PO_2 still decreases and reaches $\approx$2 mmHg. More recently, Masschelein et al. showed that muscle oxygenation, assessed by near-infrared spectroscopy, was modestly but significantly reduced ($\approx$5 %) in subjects breathing 10.7 % O_2 [7]. In the same work, tissue oxygenation index was decreased just after a moderate intensity exercise performed in normoxia ($\approx$6 %) and this decrement was slightly enhanced in hypoxia ($\approx$9 %). Mitochondrion is the final destination of O_2 into muscle cells. In skeletal muscle fiber, the PO_2 at which mitochondrial metabolism becomes inhibited is about 1.25 mmHg [8], that is far lower to what it could be encountered in hypoxic resting muscle. Altogether, it can be concluded that exercise per se induces significant reduction of muscle cell PO_2 and this diminution is worsened by hypoxic environment. Secondly, the effect of hypoxic exposure seems less potent than muscle work to decrease cellular oxygenation, although the knowledge on how hypoxia alters muscle PO_2 at rest is minimal. In tumors, it has been proposed that expression of hypoxia-regulated genes increases at PO_2 below 10–15 mmHg. Energy metabolism would be impaired at PO_2 less than 10–8 mmHg, and apoptosis would be stimulated at less than 1 mmHg [9, 10]. Skeletal muscle is probably more resistant to low O_2 levels as ATP production is maintained during exercise and it is currently not possible to set a value that would mark the occurrence of cellular hypoxic state. One can only point that long term residency of humans seems to be critical beyond 5500 m above sea level, suggesting that whole body oxygenation becomes critical for chronic exposure at this altitude.

Table 1 Correspondence between the inspired O_2 fraction (FIO_2), the altitude level and the ambient O_2 pressure

FIO_2 (%)	Altitude (m)	Ambient PO_2 (mmHg)	Inspired PO_2 (mmHg)
20.9	0	159	149
15	2700	114	107
12	4400	91	86
10	5800	76	71
8	7400	61	57
6	9400	46	43
1	19,500	8	7

The reduction of PO_2 between ambient and inspired air is due to the saturation with water vapor in the upper airway

Hypoxia-inducible factor (HIF) activation

The best way to characterize muscle cell hypoxia is to look at the cellular response. Since the article of Wang and

Semenza in 1992 [11], Hypoxia inducible factor (HIF) 1 has been described as the master regulator of hypoxia-mediated cellular adaptations. This α/β heterodimeric transcription factor regulates more than 100 genes involved in erythropoiesis/iron metabolism, angiogenesis, vascular tone, matrix metabolism, glucose metabolism, cell proliferation/survival or apoptosis [12]. Accordingly, *Hif1a*$^{+/-}$ heterozygous mice (total deletion is lethal [13]) exhibit impaired physiological responses to chronic hypoxia, including alterations in erythropoiesis, pulmonary vascular remodeling and ventilatory adaptations [14, 15].

While HIF-1β (also known as ARNT) is constitutively expressed, HIF-1α protein is quickly degraded in normoxic conditions (half-life <1 min [16]). This degradation is mediated via hydroxylation of proline residues on HIF-1α by prolyl-hydroxylases (PHD) leading to its binding to the von Hippel-Lindau (pVHL) tumour suppressor, ubiquitinylation and subsequent degradation by the proteasome (Fig. 1). A similar mechanism involving asparagine residue hydroxylation by Factor Inhibiting HIF (FIH) silences HIF-1α transactivation domain through inhibition of the transcription coactivators (p300 and CBP) recruitment in normoxia [17]. PHD-dependent regulation of HIF-1α implies the presence of O_2 and 2-oxoglutarate as substrates and Fe^{2+} as co-factor. Under reduced O_2 pressure, PHD are inhibited because of the lower O_2 availability, but also via the conversion of Fe^{2+} into Fe^{3+} due to mitochondrial reactive oxygen species (ROS) production from the complex III of the electron transport chain [18]. Consistently, data from Chaudhary et al. [19] show a strong relation between HIF-1α protein content and free radical generation in hypoxic rat muscle (Fig. 2). Inhibition of PHD results

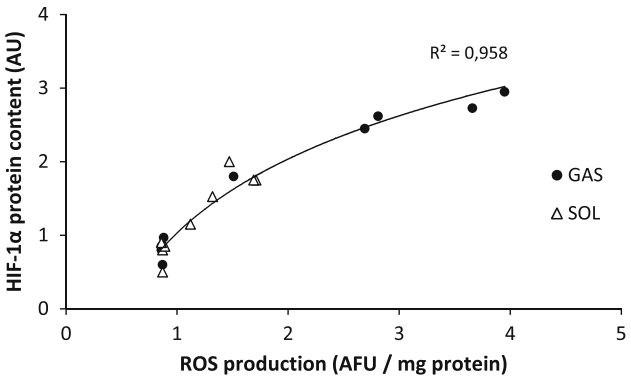

Fig. 2 HIF-1α protein content correlates with free radical generation in skeletal muscle of rats exposed to extreme hypoxia. Rats were exposed for 3, 7, 14 or 21 days to a simulated altitude of 7620 m. Higher rate of ROS generation was measured after 14 days in hypoxic environment. *GAS* gastrocnemius, *SOL* soleus. Redrawn from [19]

both in the stabilization and the dimerization of HIF-1α with the β subunit. Accordingly, hypoxia can be mimicked by iron chelation (e.g. deferoxamine), some transition metals (e.g. cobalt) or 2-oxoglutarate inhibitors (e.g. DMOG), and this property has been widely used to hypoxia preconditioning. While HIF-1α is subjected to other post-translationnal regulations such as acetylations, phosphorylations or nitrosylations (reviewed in [20]), inhibition of hydroxylation is the main regulation involved in hypoxia-induced HIF-1α stabilization.

A specificity of muscle cells could reside in HIF-1α nuclear translocation. Inhibition of HIF-1α degradation by $CoCl_2$ is sufficient to cause its nuclear accumulation in COS-7 cells, whereas this translocation requires low oxygen levels in primary myotubes. The nuclear import of HIF-1α

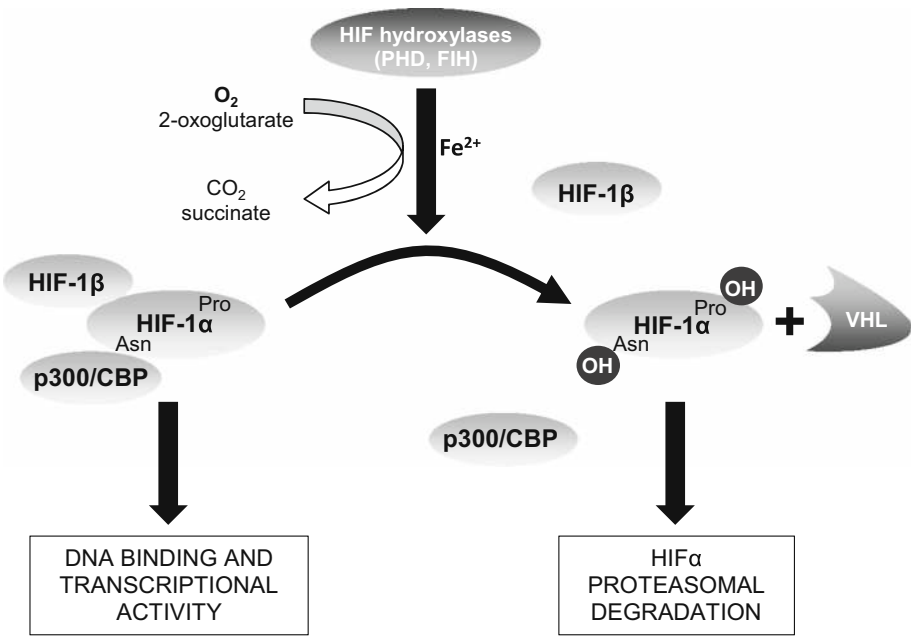

Fig. 1 Regulation of HIF-1α under normoxia and hypoxia. HIF-1α associates with HIF-1β and p300/CBP, binds DNA and activates transcription under reduced O_2. In presence of O_2 and 2-oxoglutarate, prolyl-hydroxylases (PHD) and factor inhibiting HIF (FIH) hydroxylate HIF-1α on prolines (Pro) 402 and 564 and on asparagine (Asn) 803, respectively. Asparagine hydroxylation (OH) inhibits the association between HIF-1α and p300/CBP, while prolines hydroxylation results in the binding to the E3 ubiquitin ligase Von Hippel-Lindau complex (VHL), leading to its degradation by the proteasome

in muscle cells is concomitant with its dissociation from HSP90, a protein that has been proposed to mask the nuclear localization signal of HIF-1α under normoxia [21]. Nuclear accumulation of pVHL has been observed in C2C12 myotubes exposed to 1 % O_2 and this translocation promotes HIF-1α stabilization and transcriptional activity in 117 and 786-0 cells [22]. This mechanism was not induced by the decrease in PO_2 per se, but rather results from the acidosis due to the enhancement in anaerobic glycolysis. This is of particular interest as skeletal muscle can experience metabolic acidosis during intense exercise. It should be noted that HIF-1α can also be activated by growth factors or cytokines such as angiotensin II, insulin, Insulin-like growth factor (IGF), interleukin-1 or transforming growth factor α under normoxic conditions (reviewed in [12]). Intensive researches are engaged to decipher the cross-talk between HIF-1 and inflammation notably in the fields of cancer, obesity and metabolic disorders. Some works showed normoxic HIF-1α activation through a Tumor Necrosis Factor-α/Nuclear Factor -kappa B axis [23], although the few data available in muscle cells do not support such a regulation [24]. As during hypoxia, ROS seem to play a key role for normoxic HIF-1α activation [12].

The HIF family also comprises HIF-2 and HIF-3. HIF-2α protein is subjected to similar regulation by hypoxia than HIF-1α and it is implicated in hypoxic response as it may confer muscle resistance to ischemia [25]. Yet, muscle HIF-2α protein accumulation is low 6 h after ischemia and undetectable after endurance exercise [25, 26]. Moreover, it seems to have opposite effects on muscle phenotype compared to hypoxia and HIF-1α activity (cf. below and [27]). Very few data exist on HIF-3 function and there are no studies in skeletal muscle. It has been shown that HIF-3α alternative splicing triggers it transcriptional activity on Hypoxia Response Elements [28] and one splice variant can bind and inhibit HIF-1α activity [29]. Collectively, these results suggest that skeletal muscle adaptations consecutive to hypoxic exposure are mainly driven by HIF-1, though others transcription factors could be also activated during hypoxia and thus contribute to the whole adaptative response [30]. Microarray on wild-type and HIF-1α knockout mouse embryonic fibroblasts (MEF) supports this hypothesis, since 89 % of the genes induced after exposure to 1 % O_2 were regulated by HIF-1 [31].

Hypoxic stabilization of HIF-1α in skeletal muscle: myth or reality?

The characterization of HIF-1 response to hypoxia in vivo is limited because of technical outcomes. Extrapolation of results obtained from cultured cells to whole body is subjected to caution. Cell culture experiments use extreme conditions, as control cells could be hyperoxic (20.9 % O_2 means an ambiant PO_2 of 159 mmHg that is far greater to that of capillaries) and the reduction in O_2 availability is often drastic (1 % O_2 in ambient air equal a PO_2 of 7.6 mmHg and probably less into the cells). Cell line (e.g. cancer vs. non cancer cells) is also of particular importance since it influences O_2 availability into the medium. Some cell lines are thus hypoxic at 20.9 % O_2 because of high cell density and metabolic rate [32]. This may explain why hypoxia exposure results in a modest increase in HIF-1α protein expression in L6 rat myoblasts (0.5 % O_2) or primary rabbit myotubes (3 % O_2) [21, 33].

Another major limitation is the short half-life of HIF-1α that would theoretically impact (decrease) its protein content during tissue removal or cell harvest since they are generally performed under normoxic conditions. Recent advances have been made on this issue by using live cell imaging under hypoxia. For example, Bagnall et al. demonstrated that HIF-1α protein is only transiently stabilized (3 h pulses) in HeLa cells exposed to 1 % O_2 [34]. The authors showed that the short stabilization of HIF-1α is due to the up-regulation of PHD2 (while HIF-2α seems rather regulated by PHD1 [25]). When HIF-1α protein expression is prolonged over this time lapse, cell engaged into a p53-related apoptosis program, although whether the same is true for muscle cells remains to be determined. It would be thus interesting to determine whether or not muscle PHD2 content increases during chronic hypoxia. This mechanism is likely because endurance trained subjects, whose muscles are submitted to intense hypoxic episodes during contractions, have increased PHD2 and FIH protein content in their vastus lateralis muscle [35]. Cigarette smoke exposure also increases PHD2 and pVHL protein expression in mouse skeletal muscle, concomitantly with reduced HIF-1α content [36]. One may suppose that muscle HIF-1α expression will return to basal level within a few hours even if the hypoxic environment persists as it has been observed in brain, liver and kidney [37]. Cell culture experiments support this assumption, since HIF-1α protein peaked between 4 and 24 h of exposure to hypoxia (1–2 % O_2) before returning to modest or undetectable levels in human myoblasts [38] and human induced pluripotent stem cells [39]. This could explain why gastrocnemius HIF-1α level was not increased in erytropoietin-deficient mice with an hematocrit of 30 % or in wild-type animals after 24 h or 14 days at 4500 m [40]. Skeletal muscle may also be low responder to reduced O_2 delivery, as HIF luciferase reporter activity did not increase after acute anemia in skeletal muscle contrary to other organs such as brain, kidney or liver [41] and skeletal muscle deoxygenation was half the size of brain in subjects exposed to 12 % O_2 for 4 h [42]. A last point that affects data about HIF-1α protein expression is the cellular compartment used for western blot analysis. While HIF-1α

protein is usually undetectable in total muscle lysate, the nuclear fraction contains high amounts of HIF-1α under normoxia [43], notably compared to others tissues [37]. Isolating the nuclear fraction would thus be helpful to characterize HIF-1α protein response to reduced O_2 availability.

In summary, hypoxia may be defined as a condition of low O_2 pressure sufficient to promote HIF-1α accumulation in the cells. A key signal for stabilizing HIF-1α is the increase in ROS production, even more than the decrease in O_2 pressure per se. Marked increase in HIF-1α protein content has been observed in skeletal muscle during endurance exercise and/or blood restriction [26]. However, the stabilization of HIF-1α after systemic hypoxia (e.g. altitude exposure) has not been systematically reported [40, 44] and seems rather modest (reviewed in [45]), except for severe hypoxia: FIO_2 of 6 % [37] or simulated altitude of 5800 m [46] and 7620 m [19]. Skeletal muscle HIF-1α accumulation likely reflects severe impairment of whole body oxygenation and it would be of clinical interest to measure HIF-1α accumulation in skeletal muscle of patients suffering from pathologies associated with hypoxemia (reduction in arterial O_2) or decreased blood flow. Another explanation for the lack of muscle HIF-1α accumulation under hypoxia is the transient nature of its stabilization. This raises an intriguing issue: how such a short up-regulation could have so durable consequences on muscle phenotype?

Skeletal muscle metabolic adaptations in response to chronic hypoxia

Pioneering work from Reynafarje in 1962 reported an increase in myoblogin content and higher cytochrome c reductase activity in skeletal muscle of highlanders, suggesting that prolonged hypoxia resulted in an enhancement of skeletal muscle oxidative capacity. However, further works that studied the effect of hypoxia independently from other factors, such as cold or exercise, showed that hypoxia per se promoted oxidative-to-glycolytic metabolic shift (see Table 2). Accordingly, expression of HIF-1α is higher in glycolytic vs. oxidative muscles [47] and subjects carrying a mutation that leads to increased HIF-1α stability have higher distribution of type II fibers [48]. This effect is related to the role of HIF-1 on muscle metabolism through several coordinated mechanisms.

Hypoxia-mediated regulation of metabolic pathways

HIF-1 up-regulates the expression of 11 glycolytic enzymes (aldolase A, aldolase C, enolase 1, glyceraldehyde-3-phosphate dehydrogenase, hexokinase 1, hexokinase 2, lactate dehydrogenase A, phosphofructokinase L, phosphoglycerate kinase 1, pyruvate kinase M, and triosephosphate isomerase) contributing to enhance the glycolytic capacity of cells [49]. Although these targets have often been identified in non-muscle cells, significant up-regulation of lactate dehydrogenase and hexokinase activity has been observed in skeletal muscle after hypoxia exposure (Table 2). Further analysis of the literature reveals that most activities of muscle glycolytic enzymes remain unchanged by hypoxia as recently reviewed [50]. Given that the glycolytic machinery can support strong increase in glycolysis at exercise in normoxia, one may suppose that there is no need to further upgrade the maximal capacity of this system under hypoxia (at least at rest). Muscle glycolytic capacity (assessed by lactate production under anoxia) was not enhanced after 1, 16 or 90 days at 5500 m [51], confirming the marginal effect of chronic hypoxia on the anaerobic pathway in skeletal muscles of sedentary rats.

Besides, HIF-1 inhibits oxidative metabolism by restricting the entry of pyruvate into the tricarboxylic acid cycle [52], through the induction of the pyruvate dehydrogenase kinase (PDK) 1. PDK1 phosphorylates and inactivates the pyruvate dehydrogenase enzyme complex that converts pyruvate to acetyl-coenzyme A in the mitochondria. In muscle cells, PDK1 up-regulation has been reported in rats exposed to 10 % O_2 for 2 weeks [46] and in human satellite cells treated with PHD inhibitor [35], but not in humans after 7–9 days at 4560 m [44]. This could be related to the kinetics of gene expression since 24 h of exposure to 4300 m was associated with increased PDK1 protein content while it returned to basal level after 1 week [53].

As a consequence of these regulations, muscle lactate production would be increased by hypoxia exposure. Yet, lactate concentration and lactate-to-pyruvate ratio were not elevated in the vastus lateralis of subjects exposed for either 4 h or 3 weeks at 4300 m [54, 55], while lactate accumulation was increased in the media of C2C12 myotubes 96 h after treatment with PHD inhibitor [35]. Skeletal muscle lactate efflux is notably mediated by MCT4, a monocarboxylate transporter that is upregulated during in vitro hypoxia in a HIF-1-dependent manner [56]. However, in vivo studies provided equivocal data with no change, increase or decrease in MCT4 expression [57–59]. Although extensively studied at exercise, regulation of lactate metabolism under reduced O_2 availability is not yet fully elucidated.

HIF-1 also triggers oxidative metabolism by impairing fatty acid oxidation and mice lacking skeletal muscle HIF-1α display increased lipid oxidation [60]. HIF-1 reduces the DNA binding activity of peroxisome proliferator-activated receptor α (PPARα)/retinoid X receptor complex, a major regulator of lipid oxidation [61–63]. Hypoxia also inhibits the PPARγ coactivator 1α (PGC-1α) pathway in a HIF-1-dependent way in C2C12 myotubes [64]. Along with the reduction in the transcriptional activity of the

Table 2 Effect of hypoxia on the activity of metabolic enzymes in human (H), mouse (M) or rat (R) skeletal muscles

Altitude (m)	Duration (days)	Specie	Muscle	Enzymatic activity			References
				Increased	Unchanged	Decreased	
2100	14	H	GAS		**LDH**	CS 13 %, HAD 10 %	[141]
			TB		**LDH**, CS, HAD		
3700	63	R	SOL	CS, **PFK**	HAD, **HK**, **LDH**		[142]
	63	R	PLA		**HK**, **LDH**, **PFK**	CS, HAD	
	63	R	QUA		CS, HAD, **HK**, **LDH**	**PFK**	
3800	7	M	Lower hindlimb		CS, HAD	CPT 50 %	[67]
4000	35	R	SOL		CS	HAD 51 % CPT 34 %	[86]
	84	R	SOL	**HK 40 %**	CS, **LDH**	HAD 10 %	[143]
	84	R	EDL	**LDH 10 %**	CS, **HK**	HAD 16 %	
	84	R	PLA	**HK 39 %, LDH 18 %**	CS	HAD 12 %	
4100	56	H	VL		**LDH-H**		[57]
4300	18	H	VL	**HK 19 %**	**GP**, **LDH**, MDH		[144]
	21	H	VL	**HK 16 %, PFK 13 %**	HAD, SDH		[55]
	35	R	EDL		CS	CPT 62 %	[145]
	35	R	SOL		CPT		
	35	R	QUA			HAD 31 %	
4878	28	R	SOL		**LDH, PK, HK,** CS, MDH		[146]
	28	R	PLA		**LDH, PK, HK,** CS, MDH		
5350	70	H	VL			COX, CS	[147]
5500	12	R	PLA			CS 13 %, COX 41 %	[65]
	56					CS 16 %, COX 36 %	
	21	R	PLA	**LDH-H 26 %**	**LDH (9 %), LDH-M**		[59]
			SOL	**LDH 38 %, LDH-M 100 %**	**LDH-H**		
	90	R	GAS		**LDH**		[51]
5800	14	R	GAS	**PK**	CS	MDH	[46]
	14	R	GAS			COX 55 %, CS 73 %	[148]
	21	R	SOL	**HK 44 %**	CS, HAD, **LDH**, **PFK**, **PK**		[149]
	21	R	GAS		CS		
6000	18	H	VL	**LDH 13 %**	CS		[150]
6100	7	R	GAS	**LDH 54 %**	CPT, CS (−19 %), HAD (−14 %), MDH, SDH		[66]
8848	40	H	VL		HAD, **LDH**, **PFK**, **PK**	CS 29 %, **HK 38 %**, SDH 28 %	[91]

Glycolytic enzymes are in bold and enzymes specific from lipid oxidation are underlined. The percent changes (increase or decrease) are indicated when available

Muscles: *EDL* extensor digitorum longus, *GAS* gastrocnemius, *PLA* plantaris, *QUA* quadriceps, *SOL* soleus, *TB* triceps brachii, *VL* vastus lateralis

Enzymes: *COX* cytochrome oxydase, *CPT* carnitine palmitoyl transferase, *CS* citrate synthase, *GP* glycogen phosphorylase, *HAD* hydroxyacyl-CoA dehydrogenase, *HK* hexokinase, *LDH* lactate dehydrogenase (H or M: heart or muscle isoform), *MDH* malate dehydrogenase, *PFK* phosphofructokinase, *PK* pyruvate kinase, *SDH* succinate dehydrogenase

PPAR/PGC-1α complex, hypoxia can also result in a decrease in PPARα and PGC-1α protein content [63, 65], although there are some discrepancies regarding PGC-1α expression (e.g. protein vs. mRNA levels [65]). Since PPARα and PGC-1α are major regulators of fatty acid metabolism, hypoxia leads to a decrease in mitochondrial

import and β-oxidation of fatty acids. This is notably illustrated by a decrease in carnitine palmitoyl transferase and hydroxyacyl-coenzyme A dehydrogenase activity that has frequently been reported (Table 2). The overall effect of hypoxia logically resulted in a marked reduction of fatty acid oxidation after 1 week in hypoxic environment [66, 67].

Mitochondrial remodelling and regulation of ROS production

Mountaineers which participated to expeditions in the Himalayas (Lhotse or Everest) presented diminished mitochondrial content [68], with subsarcolemmal mitochondria being more affected than the intermyofibrillar subpopulation [69]. People living permanently at high altitude (Tibetans and Quechuans) have also reduced mitochondrial content in skeletal muscle suggesting that this phenotype is conserved with long term adaptation [70]. Cellular models demonstrated that hypoxic exposure causes mitochondrial autophagy in an HIF-1-dependent way [71]. Autophagy is a process involving the formation of autophagosomes and this mechanism is initiated by the dissociation of the Beclin1/Bcl-2 complex. HIF-1 mediates the increase in BNIP3 expression during hypoxia exposure in skeletal muscle [72–74]. BNIP3 competes with Beclin1 for binding to Bcl-2 leading to the release of Beclin1 [75], thus promoting the initiation of autophagy and mitophagy. This process could also be initiated by activation of the metabolic sensor Sirt1, whose expression is increased during hypoxia in a HIF-dependent manner in Hep3B or HT1080 cells [76]. Activation of mitochondrial autophagy through a Sirt1/BNIP3 pathway attenuated hypoxia-associated renal damage [77]. This suggests that the removal of altered mitochondria is required to limit oxidative damages. A recent study showed that the mitochondrial ubiquitin ligase 1 (Mul1) targeted the mitochondrial fusion protein mitofusin-2 resulting in mitophagy during muscle wasting [78]. Whether or not Mul1 expression is increased during hypoxia remains however to be elucidated. Taken together, all these mechanisms reduce the metabolic contribution of mitochondria and increase the involvement of the glycolytic pathway to energy supply (Fig. 3). In agreement, skeletal muscle specific deletion of HIF-1α results in an increase in oxidative capacity and mitochondrial density, a reduction in serum lactate concentration and enhanced performances during endurance exercise [79]. The physiological significance of this shift toward glucose utilization is a decrease in O_2 reliance since (1) stimulation of glycolysis flux increases anaerobic ATP synthesis and (2) glucose rather fatty acid oxidation is advantageous when looking at the P/O ratio (i.e. the ATP produced relative to the amount of O_2 consumed).

Another major point concerning the issue of hypoxia-mediated mitochondrial remodelling is the regulation of ROS production. Reduced O_2 availability promotes ROS

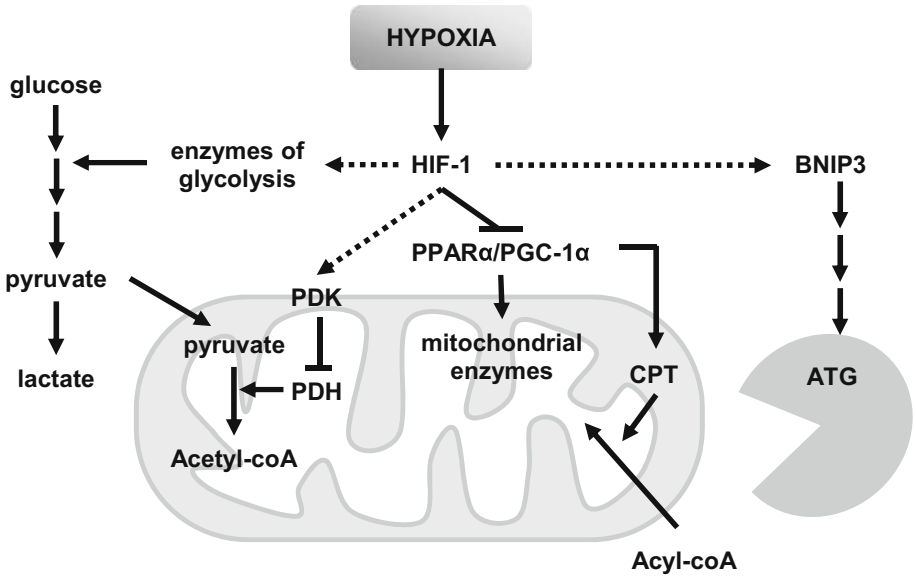

Fig. 3 Metabolic adaptations of skeletal muscle cell exposed to hypoxia. HIF-1 coordinately regulates muscle metabolism through (1) enhanced transcription of glycolytic enzymes, (2) repression of lipid oxidation via inhibition of PPAR/PGC-1α axis and (3) reduction in mitochondrial content by induction of the BNIP3-dependent mitophagy. *ATG* mitochondrial autophagy, *BNIP3* BCL2/adenovirus E1B 19kDa protein-interacting protein 3, *CPT* carnitine palmitoyltransferase, *HIF-1* hypoxia-inducible factor 1, *PDH* pyruvate dehydrogenase, *PDK* pyruvate dehydrogenase kinase, *PGC-1α* PPARα coactivator 1-α, *PPARα* peroxisome proliferator-activated receptor α. *Dotted arrows* represent transcriptional regulations

formation, and excessive ROS production represents a risk of oxidative damage that could lead to cell death if not corrected. Hypoxia exposure therefore results in cellular adaptations to counteract oxidative stress-induced damages. The importance of regulating ROS production under hypoxia was evidenced by the use of HIF-1α deficient MEFs. These cells died after 72 h in hypoxic environment due to a dramatic increase in ROS levels contrary to control cells that reduced H_2O_2 production [52]. At the mitochondrial level, O_2 is used by the cytochrome c oxidase (COX) complex. HIF-1 has been shown to up-regulate the expression of both COX4-2 subunit and LON, a mitochondrial protease targeting COX4-1 in cultured cells [80]. This switch from COX4-1 to COX4-2 leads to the improvement in the electron transfer and thus reduction in $O_2^{\cdot-}$ production. Increased UCP3 expression has also been reported after hypoxia exposure [81, 82], and this also contributes to impair ROS production. The miRNA miR-210 is a direct target of HIF-1 and its deletion enhances ROS production and decreases survival upon H_2O_2 treatment of C2C12 myotubes [83]. These adaptations together with the degradation of damaged mitochondria by autophagy would thus decrease ROS production. As ROS are involved in HIF-1α stabilization (see above), this reduction would suppress HIF-1α accumulation consistently with its transient up-regulation observed in vitro [34]. Muscle proteome of highlanders is characterized by reinforced protection against ROS compared to control subjects permanently residing at low altitude [84], and hypoxia preconditioning by $CoCl_2$ in rats reduced exercise-induced skeletal muscle oxidative damages [85]. Altogether, this confirms that regulation of redox status under reduced O_2 availability is of key importance.

Mitochondrial function is depreciated in rodents exposed to simulated altitude, even when O_2 consumption was normalized to mitochondrial protein content [86–88]. Unexpectedly, mitochondrial respiration was only modestly altered in humans exposed to 4550 m with slight reduction in maximal oxidative phosphorylation capacity [89]. This result is all the more surprising in that O_2 consumption was expressed per mg of muscle, which therefore does not take into account the potential decrease in mitochondrial density. Another difference between this latter study and the formers is that it was performed on permeabilized fibers and not on isolated mitochondria. Since this in situ approach appears to be more relevant from a physiological view, it would be interesting to confirm this result.

Skeletal muscle vascularization

Oxidative metabolism of muscle cells is tightly related to blood perfusion and muscular capillarity. Capillarization, as measured by the capillary density, is substantially and consistently increased in skeletal muscles of both humans and rats after exposure to hypobaric hypoxia of long duration [90–92]. An increase in muscle capillarity could be explained by the transcription of the Vascular Endothelial Growth Factor (VEGF) by HIF-1α. VEGF has even been characterized as "the" gene inducible by hypoxia and/or HIF-1α. Overexpression of both HIF-1α and HIF-2α promotes angiogenesis in mouse heart and rabbit ischemic skeletal muscle [93]. However, accumulating evidence suggest that VEGF mRNA is not systematically elevated throughout the exposition to hypoxia and may be even decreased after chronic exposure [94]. No study evidenced an increase in the number of capillaries per fiber in sedentary muscles e.g. [68, 90–92, 94–97] and it is now admitted that ambient hypoxia per se does not promote capillary neoformation [45, 98]. The reason of the potential higher capillary density resides in the reduction of the fiber cross-sectional area, as hypoxia may cause skeletal muscle atrophy, resulting in an enhanced capacity for oxygen diffusion.

Regulation of muscle mass

Structural modifications

The negative effect of altitude on muscle mass has been objectivized since the 80's. While this negative influence was thought to be primarily related to cold exposure, high energy expenditure and especially hypophagia [99], we and others have demonstrated that hypoxia per se accounts for skeletal muscle atrophy [74, 100, 101]. Although numerous differences exist between studies (altitude level, normobaric vs. hypobaric hypoxia, exposure duration, age of animals and acclimatization duration), analysis of published data clearly shows a significant positive correlation between hypoxia severity and the level of muscle atrophy in rats (Fig. 4a). Of note, muscle atrophy occurs at altitude higher than 5000–5500 m. One may suppose that oxidative muscles would be more affected by hypoxia as they are highly dependent from O_2 delivery, although glycolytic ones are generally more sensitive to atrophy [102–104]. Based on the Fig. 4a, it is however not clear whether muscle typology impacts susceptibility to hypoxia (see also [105]). Analysis of changes in the mean fiber cross-sectional area confirms the worsening of muscle atrophy under severe hypoxia (Fig. 4b). Type II fibers appear to be greatly affected by hypoxia, as recently suggested [106], although this assumption is dampened by the heterogeneity of data. A greater susceptibility of type II fibers to hypoxia may be explained by an already low O_2 diffusion capacity resulting from high cross sectional area and poor capilarization.

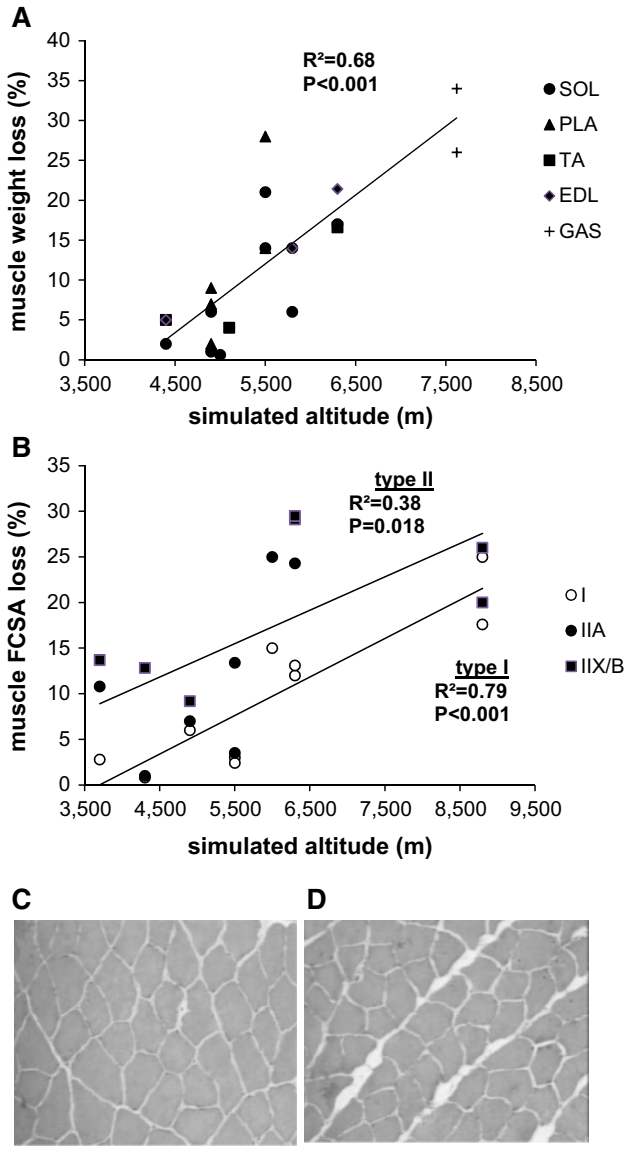

Fig. 4 Skeletal muscle atrophy in rats and humans exposed to simulated altitude between 1 and 6 weeks. **a** muscle weight (rats only) and **b** fiber cross-sectional area (FCSA) reduction (rats and humans) in function to simulated altitude level (hypobaric and normobaric hypoxia). Type IIX/B fibers measured at 8848 m are indeed undifferentiated type II fibers. **c**, **d** representative pictures from hematoxylin-eosin stained cross sections of rat plantaris muscle **c** in normoxia or **d** after 12 days at 5500 m (obtained from [118] with permission). Data for muscle weight and FCSA were obtained from [19, 101, 118, 123, 146, 149, 151–155] and [55, 91, 96, 101, 142, 151, 153, 155], respectively

Assuming that myofibrillar proteins represent about 85 % of the fiber volume [107], the regulation of skeletal muscle mass is closely related to dynamic balance between protein synthesis and degradation. Cellular experiments reveal that a marked reduction in O_2 availability decreases the rate of protein synthesis in various cell lines including C2C12 myoblasts [108]. In rats, Preedy et al. observed a non-significant decrease in muscle protein synthesis (14–17 %) after 6 h at 10 % O_2 [109]. Data in humans are scarce and are often biased by confounding factors such as cold exposure or increased physical activity (trekking) in the days preceding the analysis [110, 111]. Etheridge et al. showed that acute hypoxia had no effect on fractional synthesis rate of myofibrillar proteins at rest, whereas it blunted the anabolic effect of resistance exercise [112]. Interestingly, protein synthesis level was correlated to arterial saturation suggesting that severe hypoxemia would affect muscle anabolism.

O_2-sensitive signaling in the control of muscle mass

Activity of the Akt/mTOR pathway is a critical regulator of muscle mass by regulating protein synthesis (via the mTOR complex 1: TORC1) and proteolysis [113, 114]. We reported a down-regulation of Akt/mTOR pathway after hypoxic exposure in rats [101]. One explanation of this inhibition could be a reduction in insulin-like growth factor (IGF) 1 expression, the upstream regulator of Akt/mTOR pathway. Nevertheless, IGF-1 mRNA or protein do not present significant variations under sustained hypoxia [19, 101]. We recently observed that hypoxia blocked IGF-1-mediated activation of the Akt/mTOR pathway in C2C12 myotubes [115]. On the contrary, the expression of myostatin, a muscle-secreted factor which reduces muscle mass through inhibition of the Akt/mTOR pathway [116, 117], is increased by hypoxia exposure [106, 118, 119]. Inhibition of myostatin signaling (antibody administration, receptor inhibition or genetic ablation) partly prevents muscle atrophy under hypoxic conditions, further demonstrating that myostatin contributes to hypoxia-mediated muscle wasting [105, 119]. Loss in food intake due to hypoxia could also increase glucocorticoids pathway with the induction of KLF15 expression [106], a glucocorticoid receptor target involved in amino acid degradation to provide carbohydrate backbones for gluconeogenesis. Other circulating factors such as cytokines or adipokines may be released during hypoxia exposure, notably because of the loss of adipose tissue. However, analysis of blood cytokines profile [e.g. Tumor Necrosis Factor-α, Interleukin (IL)-1β, adiponectin, chemokine (C–C motif) ligand 5, monocyte chemoattractant protein-1] did not reveal any marked alteration with hypoxia, with the exception of an increase in IL-6 content [120]. From this study, cytokines do no seems to be implicated in hypoxia-induced muscle atrophy, although further work is required to decipher their influence on muscle phenotype.

The Akt/mTOR pathway is also regulated by AMP-activated kinase (AMPK), which causes TORC1 inhibition [121]. The α catalytic subunit of AMPK is activated when cellular energy levels are low and stimulates processes that generate ATP (fatty acid oxidation), while inhibiting those

that use ATP (triglyceride and protein synthesis). In subjects exposed to 11.5 % O_2 for 20 min, Wadley et al. did not reported any alteration of skeletal muscle AMPKα1 activity, AMPKα2 activity, AMPKα Thr172 phosphorylation, or ACCβ Ser221 phosphorylation (a direct target of AMPK) [122]. While AMPK can be transiently activated by acute hypoxia, this activation does not seem to persist after several days at altitude [73, 101, 118, 123]. These observations are consistent with the stability of AMP, ADP and ATP concentrations after 21 days at 4300 m [55] and do not support a role for AMPK in Akt/mTOR inhibition during skeletal muscle hypoxia in vivo.

Another negative regulator of TORC1 is the stress response protein REDD1 (for regulated in development and DNA damages). REDD1 has a low level of expression in skeletal muscle under basal conditions, while its expression is strongly induced by glucocorticoid and is required for glucocorticoid-induced muscle atrophy [124]. REDD1 is a direct target of HIF-1 and its expression in skeletal muscle is increased in response to hypoxia [7, 101, 118, 125]. It would therefore be a potential candidate to explain muscle loss at altitude even if REDD1 expression does not always correlate with mTOR inhibition [7, 118]. Its biological function is likely not restricted to protein metabolism, since it localizes into the mitochondria and triggers ROS production in cultured cells [126].

BNIP3 has also been proposed to inhibit mTOR through its interaction with Rheb [127]. This mechanism still needs to be confirmed as BNIP3-mediated inhibition of mTOR has never been reproduced in skeletal muscle or during hypoxia. Protein translation could also be down-regulated in a TORC1-independent way via the inhibition of the initiation factor eIF2. While eIF2-related inhibition of protein synthesis has been well characterized in tumorigenic cells [128], the few studies investigating such a mechanism in skeletal muscle do not support this hypothesis [74, 125].

In summary, hypoxia-driven reduction in skeletal muscle protein synthesis mostly results from the de-activation of the Akt/mTOR pathway consequently to upregulation of myostatin or REDD1.

Hypoxia-related regulation of proteolytic systems

Muscle protein breakdown mainly results from activation of the ubiquitin/proteasome system (UPS) and the autophagy/lysosome pathway, both processes being controlled by the Akt/mTOR signaling. The TORC2/Akt axis triggers the expression of the E3-ligases MAFbx and MuRF1 or other components of the UPS through FoxOs cytosolic sequestration. Only few experiments investigated UPS activation under reduced O_2 conditions and results regarding enzymatic activity of the 20S proteasome remain discordant [19, 101]. Kinetics is likely of particular importance when studying these markers. Some works reported an increase in MAFbx mRNA [74, 119], while this expression in not always observed after prolonged exposure [101, 123]. The other well studied E3 ligase MuRF1 presents a similar expression pattern with an increase during the first week of hypoxia exposure [74, 123] and a return to basal level during longer exposure [101, 123]. These patterns of MuRF1 and MAFbx expression in response to hypoxia are similar to those reported during unloading and muscle inactivity [129]. Nedd4, another E3 ligase that promotes skeletal muscle atrophy, has recently been shown to be involved in hypoxic response in lung [130]. Accordingly, Nedd4 expression was increased in EDL (but not in soleus) muscle of rodents exposed to 8 % O_2 [106]. While increase in MuRF1 and MAFbx expression could be related to the semi-starvation induced by hypoxia, Nedd4 regulation seems to be only dependent from O_2 pressure. Other E3 ligases such as TRAF6, MUSA-1 or Trim32 participate to the control of muscle mass [129], but knowledge about their regulation under hypoxia is minimal.

Autophagy is stimulated via different mechanisms among which the activation of ULK1 resulting from TORC1 inhibition. Hypoxia exposure has been associated with an increase in the expression of autophagic markers such as BNIP3 [72–74] or LC3 [74]. As mentioned above, activation of muscle mitophagy under hypoxic environment seems evident. However, the involvement of autophagy in the control of muscle mass during hypoxia exposure has not been extensively described and remains to be characterized.

ATF4 [131], ATF3 [132] and IκB kinase [133] are directly regulated by PHDs in non-muscle cells and may thus play a role in cellular response to O_2 deprivation independently from the HIFs pathways. ATF4 could be of particular interest since it promotes muscle atrophy [134, 135] and its mRNA was increased in the gastrocnemius of mice exposed to severe hypoxia [74]. Activation of IκB kinase in hypoxic muscle would increase Nuclear Factor κB (NF-κB) transcriptional activity, and NF-κB is known to promote muscle wasting through stimulation of UPS-mediated protein breakdown [136]. Studying the contribution of these transcription factors to muscle remodeling during hypoxia would thus be helpful to better understand signals that trigger skeletal muscle atrophy.

A transient activation of proteolysis together with a sustained reduction in protein synthesis would be advantageous to cope with the energetic challenge induced by hypoxia. On the one hand, protein synthesis is a highly ATP-consuming process accounting for ≈25 % of the total metabolic rate [1]. On the other hand, a transient activation of proteolytic systems would promote a rapid decrease in

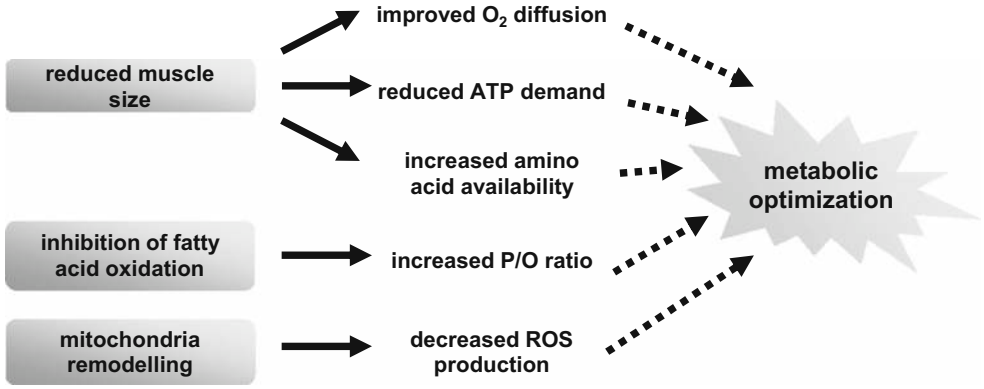

Fig. 5 Consequences of metabolic and structural adaptations on muscle homeostasis after hypoxia exposure. Reduced muscle size is mainly driven by the inhibition of the Akt/TORC1 pathway. HIF-1 inhibits the PPARα/PGC-1α axis resulting in a decrease in fatty acid oxidation. Mitochondria remodelling includes UCP3 induction, the COX-1 to COX-2 shift as well as the stimulation of BNIP3-mediated mitophagy that removes altered mitochondria

muscle mass and thus in whole body metabolic rate and the release of amino acids during muscle breakdown can also be beneficial to protect from hypoxia since they will act as metabolic fuel as well as metabolic modulator synergistically with ketone bodies [137].

Conclusion and perspectives

Hypoxia effects are primarily thought to be deleterious for skeletal muscle function, especially for mountaineers or active people who have to exercise at altitude. Nevertheless, structural and metabolic regulations that have been characterized result in metabolic optimization (Fig. 5) and are able to minimize the effects of decreased oxygen supply on muscle endurance. These effects are mainly related to HIF-1α, although it may be only transiently stabilized. Prolonged HIF-1α expression could promote apoptosis [34] suggesting that successful acclimatization needs destabilization of HIF-1α, notably via reduced ROS production. However, data about HIF-1α expression kinetics in skeletal muscle tissue of healthy subjects or patients with impaired O_2 delivery are still lacking. The exact contribution of HIF-2 in hypoxia-induced adaptations also needs to be clarified, especially as it has been proposed in cancer cells that HIF-2 is more involved in chronic adaptation to hypoxia, while HIF-1 would mediate acute responses [138]. The role of miRNAs in hypoxic response has emerged into the field of cancer research, but this remains an unresolved question concerning muscle adaptations to low O_2. Among the hypoxia-induced targets, miR-199a and −210 may be relevant candidates since they regulate muscle cell phenotype in a HIF-1-dependent way [83, 139]. Concerning experimental designs, it remains to determine whether hypobaric and normobaric hypoxia induce similar effects [140]. In addition, extrapolation of data obtained on small animals to humans requires caution because of differences in O_2 diffusion capacity. Identical PIO_2 could have attenuated effects in rodents compared to patients explaining the use of extreme hypoxia in animal studies [19, 74]. Despite the increasing number of people travelling to high altitude or suffering from pathologies associated with reduced O_2 delivery, the response of human skeletal muscle to hypoxic episodes and its interplay with other organs is still an open challenge.

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