Hypoxia and muscle maintenance regulation: implications for chronic respiratory disease

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Chiel de Theije[a], Frédéric Costes[b,c], Ramon C. Langen[d], Christophe Pison[e,f,g,h] and Harry R. Gosker[d]

[a]Nutrim School for Nutrition, Toxicology and Metabolism, Department of Anatomy and Embryology, Maastricht University Medical Centre+, Maastricht, The Netherlands, [b]Service de Physiologie Clinique et de l'Exercice, Hôpital Nord, CHU Saint-Etienne, [c]Laboratoire Physiologie de l'Exercice, EA4338 Université Jean Monnet, Saint-Etienne, France, [d]Nutrim School for Nutrition, Toxicology and Metabolism, Department of Anatomy and Embryology, Maastricht University Medical Centre+, Maastricht, The Netherlands, [e]Clinique de Pneumologie, CHU Grenoble, [f]Université Joseph Fourier, [g]InsermU1055, Grenoble and [h]European Institute for Systems Biology & Medicine, Lyon, France

Correspondence to H.R. Gosker, Department of Respiratory Medicine, Maastricht University Medical Centre+, PO Box 616, 6200 MD Maastricht, The Netherlands
Tel: +31 43 3884247;
e-mail: h.gosker@maastrichtuniversity.nl

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Purpose of review

Muscle wasting and impaired muscle oxidative metabolism are common extrapulmonary features of chronic respiratory failure (CRF) that significantly increase disease burden. This review aims to address the question whether hypoxia, an obvious consequence of this disease, actually plays a causal role in these muscle impairments.

Recent findings

In experimental models, a causal role for hypoxia in muscle atrophy and metabolic impairments has clearly been shown. Although the hypoxia-inducible factors and nuclear factor kappa B are putative mediators of these hypoxia-induced alterations, their true involvement remains to be proven. Molecular signatures of disrupted regulation of muscle mass and oxidative metabolism observed in these experimental models also have been shown in muscles of patients suffering from CRF, suggestive of but not conclusive for a causal role of hypoxia. Therapies, including but not restricted to those aimed at alleviating hypoxia, have been shown to partially but not completely restore muscle mass and oxidative capacity in CRF patients, which may imply an additive effect of nutritional modulation of substrate metabolism.

Summary

Although hypoxia clearly affects skeletal muscle maintenance, it remains to be confirmed whether and by which underlying molecular mechanisms hypoxia is causally involved in CRF-related muscle atrophy and impaired oxidative capacity.

Keywords

atrophy, chronic respiratory failure, hypoxia, metabolism, skeletal muscle

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Introduction

Muscle wasting is a common but often under-recognized extrapulmonary feature of chronic respiratory failure (CRF) that significantly increases disease burden. Loss of muscle mass can largely be attributed to muscle fibre atrophy, particularly of type II fibres [1,2]. In addition to the loss of muscle mass, peripheral muscles of patients with chronic obstructive pulmonary disease (COPD) often are characterized by a so-called loss of oxidative phenotype (OXPHEN); a shift from slow oxidative type I fibres towards fast glycolytic type II fibres, reduced oxidative enzyme capacities and mitochondrial impairments; for reviews, see [3,4]. In addition to increased fatigability, loss of muscle OXPHEN may contribute to elevated energy requirements (as oxidative energy metabolism is more efficient than glycolytic energy metabolism) and to enhanced oxidative stress, thereby augmenting the onset or progression of muscle wasting [5]. Hypoxemia, either chronic or intermittent, is an obvious feature of respiratory failure, but surprisingly its potential impact on muscle maintenance in CRF patients is rather unexplored.

Molecular sensors of hypoxia

Probably, the most important regulators of cellular responses to hypoxia belong to the hypoxia inducible factor (HIF) family of transcription factors [6]. These factors are heterodimeric proteins composed of a HIFα (HIF1–3α, of which HIF1α is best described) and a HIFβ subunit. The HIFα gene is continuously expressed, but under normoxic conditions, the protein is rapidly hydroxylated by specific prolyl hydroxylases (PHDs), enabling binding of the E3 ligase von Hippel-Lindau (VHL) leading to degradation by the ubiquitin proteasome pathway. As oxygen levels drop, so does the rate of hydroxylation, thereby allowing for the build-up of transcriptionally active HIF proteins, driving the expression of its target genes that are mainly involved in

glycolytic metabolism and angiogenesis [6]. Evidence is accumulating that the above mentioned PHDs can also modulate nuclear factor kappa B (NF-κB) signalling [7••,8], indicative of hypoxia-signalling independent of HIF1α. NF-κB is a family of proteins involved in innate immunity, inflammation and apoptosis. Its upstream regulation involves formation of an IκB kinase complex, which through its kinase activity ultimately results in NF-κB transcriptional activity [9]. Interestingly, evidence for crosstalk between HIF1α and NF-κB is also emerging [7••,8]: although HIF1α is primarily post transcriptionally regulated, NF-κB has been reported to upregulate HIF1α gene expression and *vice versa* it has been shown that HIF activates NF-κB signalling as well. Given these facts, a primary regulatory role of the HIF and NF-κB signalling pathways, orchestrated by the PHDs, seems, therefore, credible in hypoxia-induced alterations in muscle maintenance.

Hypoxia and maintenance of muscle mass

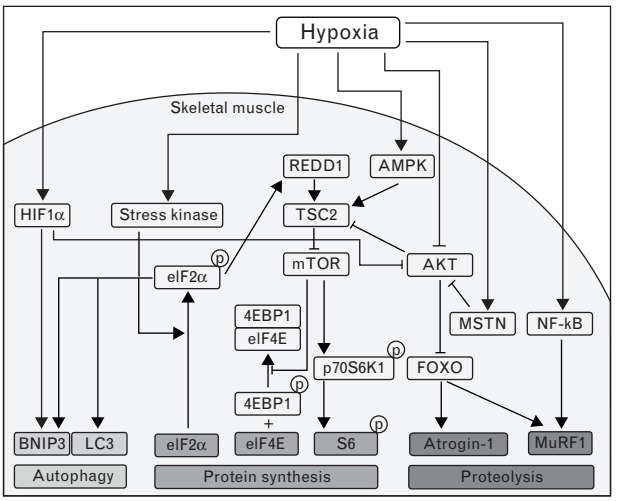
It is quite evident that hypoxia leads to muscle atrophy. Observational studies in humans showed that long-term exposure to high altitude resulted in loss of limb muscle mass, which has been described as an adaptive mechanism to improve muscle oxygenation by a relatively increased capillarization [10,11]. Muscle atrophy also occurs in animals exposed to experimental hypoxia [12•,13•]. The balance between protein synthesis and degradation is an important determinant of the maintenance of skeletal muscle mass. Although most experimental work on hypoxia-induced protein turnover has thus far focused on regulation of protein synthesis by hypoxia, effects on ubiquitin (Ub) 26S-proteasome-mediated protein degradation, as well as autophagy also have been described (see Fig. 1 for a schematic overview). As most of this work has been performed in nonmuscular cell types and tissues, extrapolation of these results to skeletal muscle protein turnover must be done with care.

Control of transcription can influence the rate of protein synthesis and can be regulated by transcription factors. HIFs are recognized as a key modulator of the transcriptional response during hypoxic stress and are involved in many adaptive responses including protein synthesis [14•]. The rate of mRNA translation is mainly controlled during the initiation phase by eukaryotic translation initiation factors (eIFs). eIF2α is permissive to mRNA translation in the nonphosphorylated state, but blocks the initiation of protein synthesis once it becomes phosphorylated by one of the four stress kinases which are, among others, activated by hypoxia [15], oxidative and endoplasmic reticulum stress [16]. Another eIF, eIF4E, is inhibited when bound by eIF4E-binding protein 1 (4EBP1). Phosphorylation of 4EBP1 by the mammalian target of rapamycin (mTOR) results in dissociation of eIF4E from 4EBP1 and the formation of the translation-initiation complex [17]. mTOR also activates ribosomal protein S6 kinase beta-1 (P70S6K1), which in turn, phosphorylates the ribosomal protein S6 (S6) and, thus, stimulates translation. Hypoxia reduces phosphorylation of mTOR and its downstream effectors 4EBP1 and P70S6, and, thus, inhibits protein synthesis [18]. mTOR phosphorylation itself is controlled by the tuberous sclerosis protein 1 and *2* (TSC1/TSC2) complex, which

Key points

- Maintenance of muscle mass and metabolism is clearly affected by hypoxia.
- The molecular mechanisms through which chronic hypoxia affects muscle maintenance are poorly understood.
- A causal role for hypoxia in muscle atrophy and impaired oxidative phenotype (OXPHEN) in chronic respiratory failure is feasible, though remains to be confirmed.
- Despite these uncertainties, therapies aimed at alleviating hypoxemia or multimodal rehabilitation strategies including nutritional modulation can, at least partially, restore muscle mass and OXPHEN in chronic respiratory failure.

Figure 1 Schematic overview of potential direct or indirect hypoxia signals with respect to the control of muscle protein turnover



Hypoxia-inducible factor 1α (HIF1α), BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3), microtubule-associated protein 1 light chain-3 (LC3), eukaryotic translation initiation factor 2α (eIF2α), DNA-damage-inducible transcript 4 protein (REDD1), tuberous sclerosis protein 2 (TSC2), mammalian target of rapamycin (mTOR), eIF4E-binding protein 1 (4EBP1), eukaryotic initiation factor 4E (eIF4E), ribosomal protein S6 kinase beta-1 (P70S6K1), ribosomal protein S6 (S6), AMP-activated protein kinase (AMPK), serine/threonine protein kinase AKT (AKT), Myostatin (MSTN), Forkhead box O (FOXO), nuclear factor kappa B (NF-κB), muscle atrophy F-box (Atrogin-1), muscle-specific ring finger 1 (MuRF1).

is regulated by hypoxia-sensitive pathways, including AMP-activated protein kinase (AMPK) and DNA-damage-inducible transcript 4 protein (DDIT4/REDD1). AMPK stimulates TSC2, which in turn inactivates mTOR [19]. The degree of AMPK activation depends on the severity of the hypoxic conditions [20]. Increased REDD1 expression during hypoxia results from increased expression of the transcription factors ATF4 and C/EBP-β. Hypoxia-induced expression of ATF4 and C/EBP-β is a result of the development of endoplasmatic reticulum stress and signalling via eIF2α and does not seem to be dependent on HIF1α [12•,20,21]. Subsequent REDD1 expression results in activation of TSC2, which subsequently inhibits mTOR in response to hypoxia [22].

AKT is a serine/threonine protein kinase and a key protein in the regulation of muscle mass, which controls protein synthesis via mTOR, but also regulates protein degradation. AKT activation by phosphoinositide 3-kinase (PI3K) is involved in the stimulation of myogenic differentiation by insulin growth factor-1 (IGF-1). During hypoxia, this myogenic response changes into a mitogenic response by redirection of IGF-1 signalling to mitogen-activated protein kinase (MAPK) instead of AKT activation. This finding implies that oxygen gradients may be of importance for myogenesis. It is currently unknown by which mechanisms hypoxia influences AKT phosphorylation, although HIF1α may play a role [14•].

Increased protein degradation in skeletal muscle results from increased lysosomal and/or proteasomal protein degradation. Autophagy can be initiated in response to nutritional depletion or hypoxia via activation of HIF1α and/or endoplasmatic reticulum stress via BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) and microtubule-associated protein 1 light chain-3 (LC3) [23,24•]. Autophagy, which captures organelles and proteins in autophagic vacuoles, relies on lysosomal protein degradation and is important in muscle maintenance [25]. Muscle protein degradation is also controlled by the ubiquitin-proteasome system (UPS). In this pathway, the E3 Ub-ligases, muscle-specific ring finger 1 (MuRF1) and muscle atrophy F-box (Atrogin-1/MAFbx), label muscle proteins with poly-ubiquitin (Ub) chains, resulting in their targeted degradation by the 26S-proteasome. The expression of both MuRF and Atrogin-1 are increased during hypoxia [26,27]. The expression of these atrogenes is regulated by inducible transcription factors like NF-κB [28] and Forkhead box O (FOXO). FOXO1 is negatively controlled by AKT-mediated phosphorylation, which results in its nuclear export and subsequent suppression of the transcription of MuRF1 and Atrogin-1 resulting in decreased protein degradation [29,30]. However, the mechanisms by which hypoxia controls expression of these atrogenes are still unclear. The regulation of AKT activity may be the key in regulating muscle mass during hypoxic conditions. Myostatin (MSTN) signalling is able to suppress AKT activation, which in turn decreases protein synthesis via mTOR and derepression of FOXO1-mediated atrogenes transcription [31,32]. MSTN expression is increased in muscle atrophy during hypoxic conditions in humans, rats and muscle cells [13•]. Protein synthesis and degradation are regulated by complex mechanisms, which allow rapid adaptation to acute hypoxic stress. However, in response to sustained hypoxic stress, adaptive mechanisms may not be adequate in maintaining the balance in muscle protein turnover, which may culminate in muscle atrophy in chronic disease.

Hypoxia and maintenance of muscle metabolism

Literature is rather inconsistent regarding the long-term response of muscle metabolic profile to hypoxia and the underlying molecular mechanisms remain unclear. The adaptations of muscle in humans exposed to high altitude have been extensively studied and the overall consensus is that muscle adapts to high altitude hypoxia by a decrease in oxidative capacity, whereas combined with exercise training, muscle OXPHEN may even improve [6,33,34]. In addition, numerous experiments have been conducted in which rodents were exposed to chronic hypoxia and in some of these studies, a relative loss of muscle OXPHEN was indeed found, but not in others. Although yet to be confirmed, it is possible that these discrepancies were caused by differences in age as only the younger animals seemed to exhibit loss of muscle OXPHEN [35]. By using an in-vitro model in which cultured muscle cells were exposed to low oxygen levels, the mere effect of hypoxia could be studied, which revealed a hypoxia-induced downregulation of the expression of mitochondrial proteins [36]. The exact mechanisms underlying hypoxia-induced loss of muscle OXPHEN also remain largely unclear. There are a few indications that point towards involvement of HIF1α, which as mentioned earlier, indisputably is an important enhancer of glycolytic metabolism, thus 'away from' oxidative metabolism. Muscles of patients with Chuvash polycythemia, a disease with a genetic abnormality resulting in an impaired HIF1α degradation resulting in elevated HIF1α levels at normal oxygen tensions, exhibited early and accelerated phosphocreatine depletion accompanied by increased acidosis and lactate accumulation during exercise, indicative of impaired muscle OXPHEN [37]. Furthermore, higher mitochondrial enzyme activities have been observed in muscles of mice lacking HIF1α [38]. Another potential way of adjusting mitochondrial capacity to hypoxia is mitochondrial autophagy, which also requires HIF1α [39]. In rats, HIF1α expression levels were found to be highest in the

fast-twitch type II glycolytic muscles [40]. This study furthermore showed elevated HIF1α levels in muscles electrically stimulated with a high frequency (inducing fast fibre type-specific genes) and reduced HIF1α levels after low frequency stimulation (inducing slow fibre type-specific genes), stressing the role of HIF1α in muscle OXPHEN regulation even independent of hypoxia. It is, however, unclear whether these results can be extrapolated to humans, as HIF1α protein expression was found not to be highest in glycolytic muscles but rather in oxidative muscles [41]. The peroxisome proliferator-activated receptors (PPARs) and in particular their co-activator PGC1α are key regulators of OXPHEN in the pre-existing and developing muscle [42••,43]. In-vitro studies showed that hypoxia can certainly impair components of the PPAR pathway at transcriptional and post-transcriptional level in cultured muscle cells [39], feasibly leading to loss of muscle OXPHEN. Controversially, PGC1α has been shown to induce the expression of typical HIF1α target genes [especially those involved in angiogenesis, like vascular endothelial growth factor (VEGF)] at physiological oxygen levels [44]. A plausible explanation for this apparent paradox might simply be the fact that PGC1α-driven mitochondrial biogenesis leads to an increased oxygen demand, which is not (yet) matched by the oxygen supply and hence intracellular hypoxia occurs triggering HIF1α-dependent gene expression [45]. Alternatively, as NF-κB also has been implicated in hypoxia-signalling, it can be speculated that NF-κB also mediates hypoxia-induced loss of muscle OXPHEN. Remels *et al.* [46] have recently shown that NF-κB activation indeed impairs muscle OXPHEN, although it remains to be established whether this also occurs under hypoxia. Recapitulated, there are clear indications that hypoxia can impair muscle OXPHEN, but it remains to be clarified under what specific conditions and through what mechanisms this really occurs.

Implications for chronic respiratory disease

Having discussed the (potential) mechanisms through which hypoxia may impair muscle maintenance, what indications do we actually have that hypoxia is involved in muscle pathology in respiratory disease? The first probably came from Jakobsson *et al.* [47] who reported low percentages of type I fibres that were associated with low arterial oxygen pressures in COPD. Impaired muscle OXPHEN in COPD has been a consistent finding since then, but strong evidence for the involvement of hypoxia is lacking simply because this has not been further studied in groups of patients with severe hypoxemia (PaO_2 <7.3 kPa). In patients with restrictive lung disease and mild hypoxemia related to scoliosis, Swallow *et al.* [48•] showed that impaired muscle function was associated with a decreased proportion of type I fibres and increased oxidative stress. Pulmonary arterial hypertension is also an important cause of chronic hypoxemia and skeletal muscle abnormalities have indeed been reported in these patients as well, including decreased type I fibre proportions and slightly reduced oxidative enzymes [49]. As discussed above, the PPARs/PGC1α are key OXPHEN regulators that may be under negative control of hypoxia. Reduced expression levels of these regulators have indeed been shown in muscles of patients with COPD [50]. Regarding muscle atrophy, there are some indications that in muscles of COPD patients, markers of the ubiquitin proteasome pathway, including the atrogenes MuRF1 and Atrogin-1, are increased [51,52]. Moreover, COPD exacerbations are frequently associated with augmented hypoxemia and fascinatingly, it has recently been shown that the gene expression of these atrogenes was upregulated, whereas OXPHEN expression was downregulated, in patients experiencing an exacerbation as compared to stable COPD patients [53•]. Finally, increased expression levels of MSTN have been reported for COPD patients characterized by hypoxemia [13•]. It is tempting to attribute all these findings to hypoxemia, but a definitive conclusion is impossible. To add to the ambiguity, the involvement of the putative hypoxia sensors HIF and NF-κB in COPD-related muscle pathology also remains distinct: muscle HIF gene expression tended to be increased in COPD, whereas the expression of the E3 ligase VHL, which targets HIFα for degradation, was also increased [54•]. Increased muscle NF-κB activation has indeed been reported in COPD patients with low body weight [55], although unaltered muscle NF-κB activation has recently been observed in patients with muscle atrophy [52]. The influence of chronic hypoxemia was more specifically studied by Favier *et al.* [12•] who reported a downregulation of the anabolic AKT/mTOR pathway and a tendency towards an upregulation of its putative inhibitor REDD1 in hypoxemic *versus* normoxemic patients with COPD.

In addition to the above mentioned direct effects of hypoxia on muscle maintenance, indirect mechanisms may also be involved in COPD. Loss of appetite and subsequent cachexia is common in advanced COPD and elevated circulating levels of leptin, a hormone that indeed attenuates appetite and is induced by hypoxia through HIF1α, have been reported for cachectic COPD patients with more severe hypoxemia [56] and for COPD patients suffering from an acute exacerbation [57]. Intriguingly, a correlation between the degree of hypoxemia and circulating tumor necrosis factor (TNF-α) in patients with COPD was observed as well [58], supportive of the occurrence of hypoxia-induced systemic inflammation [59], which in turn may contribute to muscle pathology as well.

Additional arguments in favour of a role of hypoxia in impaired muscle maintenance come from interventions

aimed at alleviating hypoxemia. Jakobsson and Jorfeldt [60] found signs of improved muscle oxidative metabolism after long-term oxygen therapy (LTOT). Lung volume reduction surgery (LVRS) represents a functional treatment for emphysema, which improves respiratory mechanics and reduces dyspnea. Mineo *et al.* [61] showed improvements in muscle mass that were maintained for at least 5 years following LVRS and were associated with improved outcomes. Interestingly, they also reported a decrease in plasmatic inflammatory markers, such as TNF-α, IL-6 and IL-8, 1 year after surgery [62••], pointing towards the pre-existence of hypoxia-induced systemic inflammation. After lung transplantation, thus despite correction of hypoxemia, only partial restoration of muscle OXPHEN has been reported, suggesting a long-lasting signature of chronic hypoxia that could partially be reversed by rehabilitation [63–65]. Indeed, also therapies not (directly) aimed at alleviating hypoxemia may prove beneficial, as for example recently shown by improved muscle mass and performance in patients with CRF and cachexia after multimodal nutritional rehabilitation [66].

Conclusion

In experimental models, hypoxia evidently leads to skeletal muscle atrophy and renders muscles less dependent on oxidative energy metabolism. With respect to the latter, it is, however, still questionable whether hypoxia actually induces loss of muscle OXPHEN or merely increases anaerobic capacity. Loss of muscle mass and OXPHEN are evident in CRF and have been associated with hypoxemia and although therapies aimed at alleviating hypoxemia have in fact been shown to partially restore muscle mass and OXPHEN, a causal role for hypoxia in the muscle impairments in CRF remains to be verified. Moreover, alternative therapies, including nutritional modulation, offer perspectives on improvement of muscle maintenance in CRF.

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Conflicts of interest

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