Valorisation
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Societal value of the thesis

Chronic obstructive pulmonary disease (COPD) is a disease characterized by irreversible obstruction of airflow in the lungs and damage of the alveolar walls resulting in reduced gas exchange. Active smoking is the main risk factor for developing COPD, but air pollution, dust and frequent respiratory infections also contribute. According to the World Health Organization, approximately 65 million people worldwide are affected by COPD, and it is estimated that COPD will be the third leading cause of death in 2020. In the Netherlands, according to Statistics Netherlands (CBS), 54,771 persons died of airway-related diseases between 2009 and 2012, of which 25,409 persons due to COPD [1]. On the 1st of January 2011, 361,800 COPD patients were registered in the Netherlands of which 32,500 patients were newly diagnosed that year [2]. COPD becomes manifest at the age of 55 and higher and the prevalence increases with age [2]. In 2010, there were 22,400 hospitalizations of COPD patients with in total 200,000 days in hospital [2].

COPD is not limited to the lungs as patients often encounter extrapulmonary manifestations such as skeletal muscle-wasting and -weakness and most patients suffer from one or more comorbidities. Muscle wasting is a predictor of morbidity and mortality [3-5]. The features of COPD, such as low-grade inflammation, malnutrition, physical inactivity, corticosteroid medication and hypoxia all contribute to the loss of skeletal muscle mass. COPD patients may experience various degrees of oxygen desaturation, which could result in tissue hypoxia. This may occur during exercise (exertional desaturation), during the night (nocturnal desaturation), during disease exacerbations or chronically due to severely impaired lung function, such as in the case of chronic respiratory failure (CRF). Hypoxemia, either chronic or intermittent, is therefore an obvious feature of COPD, but surprisingly its impact on the maintenance of muscle mass is rather unexplored. This thesis investigated whether and how hypoxia contributes to loss of muscle mass. We developed a mouse model and extensively investigated in different muscle types the effects of hypoxia on muscle maintenance.

Innovation

We extensively described a mouse model of normobaric hypoxia, in which oxygen concentrations in blood, muscle and fat mass were measured. Protein turnover regulation was investigated by focusing on the regulation of protein degradation and synthesis. As hypoxia clearly affected food intake, the inclusion of a pair-fed (PF) group allowed us to discriminate hypoxia-specific effects from effects
resulting from reduced food intake alone. Consequently, hypoxia-specific effects that accompanied additional muscle atrophy included enhanced regulation of protein degradation and surprisingly, sustained mTORC1 activity compared to mere reduced food intake. 

Interestingly, despite higher oxidative capacity of the soleus muscle, the glycolytic EDL was more sensitive to hypoxia-induced muscle atrophy. Hypoxia increased expression of hypoxia-inducing factor α (HIF1α)-, glucocorticoid receptor (GR)-, ubiquitin proteasome system (UPS)- and autophagy lysosomal pathway (ALP) signaling mainly in the glycolytic muscle in line with the reduced muscle mass. We speculated that the glycolytic muscle is more sensitive to hypoxia-induced muscle atrophy due to a lower capillary density compared to the oxidative muscle. As GR signaling and plasma corticosterone levels were elevated by hypoxia, GR signaling was further investigated. Whereas the genetic approach to study GR involvement in protein turnover regulation revealed that GR is not essential to induce protein degradation signaling by hypoxia, these studies identified GR as a nodal point in sustained mTORC1 signaling under hypoxic conditions. In addition, impaired AMPK signaling accompanied aberrantly sustained mTORC1 signaling under hypoxic conditions during fasting.

**Knowledge utilization**

Similar to other studies [6-8] we showed that hypoxia reduced food intake. Vermeeren et al. [9, 10] showed that COPD patients with exacerbations had a reduced dietary intake of ~40% when admitted to the hospital. In addition, oxygen levels in exacerbation patients have been shown to drop as low as 35% (SaO2) indicating that severe hypoxemia may occur during disease flare-ups [11]. COPD patients in general may experience hypoxic conditions during daily activities which could affect appetite and dietary intake not only during an exacerbation [12, 13]. The direct effects of oxygen treatment on nutritional intake are rather unexplored although many patients with long-term oxygen treatment (LTOT) are still malnourished based on their low body mass index (BMI) [14]. Combination of oxygen treatment with dietary and nutrient interventions may hold the key to resolve loss of BMI and muscle wasting.

The finding that hypoxia mainly affects glycolytic muscle implicates hypoxia as a putative trigger of muscle atrophy in COPD, as in COPD patients loss of muscle fiber cross section area (FCSA) is mainly seen in the glycolytic type IIX and IIA/IIX hybrid fibers [15, 16]. Furthermore, the characteristic shift from type I to II fibers observed in muscle of COPD patients, makes them even more vulnerable
to stimuli that preferentially evoke atrophy of glycolytic muscle fibers, such as hypoxia. Therefore, addition of an endurance component to physical training in addition to resistance exercise within this patient group may be beneficial as it will not only support maintenance of muscle fiber size but also composition, thereby preventing the fiber type switch to more atrophy-prone type II fibers. Currently there is little evidence to support the use of oxygen during training, due to differences in studies and low numbers of participants [17]. Therefore a large study should be commenced combining oxygen treatment with endurance training in order to prevent muscle wasting in COPD patients.

The novel finding that hypoxia impairs protein synthesis regulation is of major interest, especially since retained protein synthesis signaling is observed in nutritionally deprived conditions. This suggests that coupling of protein synthetic responses to nutritional status or other anabolic signals, is impaired under hypoxia. Such impaired coordination between anabolic response and anabolic signals by hypoxia may be responsible for the lack of response to nutritional interventions in exacerbation patients, who did not increase fat free mass (FFM) or muscle mass [9]. Therefore, this effect should further be investigated as actual proteinsynthesis rates were not measured in this thesis. Future experiments should include infusion of stable isotopes- or radioactively labelled amino acids such as phenylalanine or leucine to measure protein synthesis rates.

This thesis showed for the first time that the glucocorticoid receptor (GR) is involved in the impaired protein synthesis regulation by hypoxia, although the exact mechanism remains to be determined. As GR is a transcription factor, a more comprehensive expression analysis in WT and mGRKO muscle following hypoxia could reveal the GR-dependent gene(s) involved in the differential protein synthesis regulation. GR also has actions that do not involve transcriptional effects (non-genomic actions) through protein-protein interactions. Immuno-precipitations (IP) with GR in combination with a target protein array or mass spectrometry could identify the proteins involved in the impaired protein synthesis regulation. Involvement of GR in the impaired protein synthesis regulation by hypoxia could have implications for the treatment of COPD patients using glucocorticoids (GCs). Oral administration of high doses of GCs during an exacerbation will affect muscle mass irrespective of the hypoxic conditions. GC administration combined with the hypoxic conditions and inflammation may further aggravate muscle loss. Refraining patients from GC administration during exacerbations might avoid GC-induced muscle loss. However, in absence of the anti-inflammatory effects of GCs, inflammation-driven muscle atrophy may occure as exacerbations are often accompanied by pulmonary and systemic inflammation [18]. Use of gluco-
corticoids combined with GR antagonists, such as RU486 or RU40555, does not seem beneficial as they are not specific for skeletal muscle and may therefore affect other tissues. More interesting is the novel field of selective glucocorticoid receptor modulators (SGRMs) [19]. It is assumed that the anti-inflammatory effects of GCs are largely due to GR transrepression mechanisms, while GR transactivation is accountable for the greater part of GC treatment-associated side effects such as muscle wasting [20, 21]. SGRMs exhibit the anti-inflammatory effects but with reduced side effects, such as skin atrophy [22, 23]. Treatment of COPD patients with SGRMs instead of GCs may therefore protect muscle mass from GC induced muscle atrophy and may result in normal protein synthesis regulation under hypoxic conditions.

Fasting under hypoxic conditions revealed that impaired protein synthesis regulation by hypoxia was accompanied by disrupted AMPK activation. As key regulator of energy homeostasis, activation of AMPK in skeletal muscle leads to increased glucose uptake and elevated fatty acid oxidation [24]. AMPK is regulated by phosphorylation on for instance Threonine 172 by LKB1 in combination with allosteric modulators such as AMP and glycogen which results in a specific activity of AMPK [24]. Future research should address at what level AMPK activation is impaired under hypoxia, for instance by measuring the allosteric modulators AMP and glycogen or by evaluating activating and inhibiting phosphorylation sites of AMPK. In subsequent studies, the causal contribution of impaired AMPK activation to protein synthesis regulation and muscle atrophy in response to hypoxia should be addressed, as it may uncover therapeutic implications of AMPK modulation during hypoxia. The use of for instance AMPK activator AICAR to restore energy homeostasis and protein turnover may have clinical benefits as intervention studies in animals in muscle showed increased AMPK activity, increased glucose uptake [25], elevated glycogen concentration [26], improved mitochondrial morphology [27], enhanced running endurance by 44% [28] and fiber type shift towards oxidative fibers [29].

In addition to studying the effects on muscle mass, the normobaric hypoxia mouse model also provides a basis for investigating other aspects of muscle dysfunction in COPD, e.g. loss of oxidative phenotype (OXPHEN), as well as the pathobiology of other tissues in COPD or the role of hypoxia in other diseases. As this model results in systemic hypoxia, other tissues including brain, liver, heart, fat, lungs and intestine could be investigated to address the effects of hypoxemia and tissue hypoxia, as these may contribute to the multi organ dysfunction in COPD patients. Currently, already a couple of scientific papers are based on this mouse model: 2 papers published by Gopal et al. and Eurlings et al., which
used the mouse model to investigate the remodeling of the lungs by hypoxia [30, 31]. Furthermore, a paper by Poels et al. that used the current mouse model looked into ventricular remodeling of the heart [32]. A paper by Slot et al. [33] investigated the effects of hypoxia on the oxidative metabolism in mice of different ages and a paper by van den Borst et al. [34] showed that white adipose tissue of hypoxic mice resulted in a more brown adipose tissue phenotype. The hypoxic model can further be extended to mimic sleep apnea by intermittent hypoxia during sleep (day time).

In conclusion, this mouse model of normobaric hypoxia is a valuable tool for multiple research questions regarding hypoxia-imposed tissue dysfunction. Moreover, besides early recognition of desaturation episodes and the treatment with oxygen, COPD patients may benefit from controlled dietary and nutrient interventions. The novel findings that hypoxia impairs protein synthesis regulation with involvement of GR and AMPK should be further studied and may reveal targets for interventions and a treatment against muscle loss in COPD patients.
References


