Review
https://doi.org/10.1631/jzus.B2300680

Autophagy in skeletal muscle dysfunction of chronic obstructive pulmonary disease: implications, mechanisms, and perspectives

Xiaoyu HAN[1], Peijun LI[2], Meiling JIANG[1], Yuanyuan CAO[1], Yingqi WANG[2], Linhong JIANG[2], Xiaodan LIU[2, 3, 4]✉, Weibing WU[1]✉

[1] *Department of Sports Rehabilitation, Shanghai University of Sport, Shanghai 200438, China*
[2] *School of Rehabilitation Science, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*
[3] *Institute of Rehabilitation Medicine, Shanghai Academy of Traditional Chinese Medicine, Shanghai 201203, China*
[4] *Engineering Research Center of Traditional Chinese Medicine Intelligent Rehabilitation, Ministry of Education, Shanghai 201203, China*

Abstract: Skeletal muscle dysfunction is a common extrapulmonary comorbidity of chronic obstructive pulmonary disease (COPD) and is associated with decreased quality-of-life and survival in patients. The autophagy lysosome pathway is one of the proteolytic systems that significantly affects skeletal muscle structure and function. Intriguingly, both promoting and inhibiting autophagy have been observed to improve COPD skeletal muscle dysfunction, yet the mechanism is unclear. This paper first reviewed the effects of macroautophagy and mitophagy on the structure and function of skeletal muscle in COPD, and then explored the mechanism of autophagy mediating the dysfunction of skeletal muscle in COPD. The results showed that macroautophagy- and mitophagy-related proteins were significantly increased in COPD skeletal muscle. Promoting macroautophagy in COPD improves myogenesis and replication capacity of muscle satellite cells, while inhibiting macroautophagy in COPD myotubes increases their diameters. Mitophagy helps to maintain mitochondrial homeostasis by removing impaired mitochondria in COPD. Autophagy is a promising target for improving COPD skeletal muscle dysfunction, and further research should be conducted to elucidate the specific mechanisms by which autophagy mediates COPD skeletal muscle dysfunction, with the aim of enhancing our understanding in this field.

Key words: Autophagy; Skeletal muscle dysfunction; Chronic obstructive pulmonary disease; Mitochondria; Muscle satellite cells

unedited

1 Introduction

Chronic obstructive pulmonary disease (COPD) is a heterogenous pulmonary condition characterized by chronic pulmonary symptoms, such as dyspnea and cough, resulting from airway and/or alveolar abnormality (GOLD, 2023). In 2019, COPD ranked as the third leading cause of death worldwide (WHO, 2019). Given the aging population and the increasing number of smokers, the prevalence of COPD is expected to rise (Christenson et al., 2022). Skeletal muscle dysfunction is a common extrapulmonary comorbidity of COPD, with an incidence of approximately 20-40% (Benz et al., 2019; Maltais et al., 2014a; Vermeeren et al., 2006; Vestbo et al., 2006), which is manifested by changes in skeletal muscle structure, function, and metabolism (Jaitovich and Barreiro, 2018). Skeletal muscle dysfunction, leading to decreased respiratory function and exercise capacity, is detrimental to quality-of-life and is associated with a high risk of mortality in COPD (Fermont et al., 2019). What is more, skeletal muscle dysfunction is the independent predictor of cachexia, admission rate, and survival rate in COPD patients (Maltais et al., 2014a; Swallow et al., 2007). Therefore, it is

✉ Weibing WU, wwb75@126.com
Xiaodan LIU, hzhp403@126.com
Weibing WU, https://orcid.org/0000-0003-3151-9828
Xiaodan LIU, https://orcid.org/0000-0002-8379-0292

Received Sept. 19, 2023; Revision accepted Dec 22, 2023;
Crosschecked xxx. xx, 20xx; Published online xxx. xx, 20xx

© Zhejiang University Press 2024

imperative to address and prevent skeletal muscle dysfunction in COPD.

The main molecular mechanisms of skeletal muscle dysfunction in COPD remain unknown. Skeletal muscle dysfunction in COPD can be attributed to various factors such as decreased physical activity, oxidative stress, inflammation, hypoxia, anabolic and catabolic imbalance, muscle recovery dysfunction, and autophagy (Hussain and Sandri, 2013; Jaitovich and Barreiro, 2018; Maltais et al., 2014b). As one of the proteolysis systems, the autophagy–lysosome pathway may play an important role in COPD skeletal muscle dysfunction (Balnis et al., 2022; Gouzi et al., 2018; Hussain and Sandri, 2013; Ito et al., 2022). Autophagy sustains cellular homeostasis by eliminating long-lived proteins and impaired organelles (Wang et al., 2018). Its role in the skeletal muscle dysfunction of neurodegenerative disease and aging has been proven (García-Prat et al., 2016). In the context of COPD, in vivo and in vitro studies demonstrated that levels of autophagy-related protein, such as microtubule-associated protein light chain 3 (LC3) BII/LC3BI, myosin-like BCL2 interacting protein (Beclin1), and p62/SQSTM1 in COPD skeletal muscle are significantly elevated (Hussain and Sandri, 2013; Mano et al., 2022) and are negatively related to the 6-minute walking distance (Gouzi et al., 2018). Knockout of PARKIN, a crucial E3 ubiquitin ligase in mitophagy, made COPD mice exhibit reduced gastrocnemius muscle cross-sectional area (CSA) and grip strength (Ito et al., 2022). The diameter of cultured myotubes from COPD patients was significantly increased after the application of autophagy inhibitor 3-methyladenine (3-MA) (Gouzi et al., 2018). However, it has also been found that the systematic application of spermidine (autophagy inducer) can significantly alleviate the proliferation disorder of muscle satellite cells caused by autophagy deficiency (Balnis et al., 2022). Existing evidence has proved that autophagy-related proteins are significantly altered in COPD skeletal muscle, and the regulation of autophagy can significantly improve the structure and function of COPD skeletal muscle. However, the results are inconsistent. In order to further clarify the role of autophagy in COPD skeletal muscle dysfunction and the mechanisms, this paper reviewed recent studies on skeletal muscle autophagy in COPD, with a view to providing new targets for the treatment of COPD skeletal muscle dysfunction.

unedited

2 Overview of COPD skeletal muscle dysfunction and autophagy

2.1 Overview of COPD skeletal muscle dysfunction

COPD skeletal muscle dysfunction is characterized by structural, functional, and metabolic alterations (Jaitovich and Barreiro, 2018). Structural changes include a decrease in CSA, muscle mass, and myogenesis protein, as well as an increase in muscle degradation proteins. Studies have shown a significant decrease in mid-thigh CSA in COPD patients (Bernard et al., 1998; Roig et al., 2011), regardless of the severity of the disease stages (Shrikrishna et al., 2012). Furthermore, even when compared to age- and fat-free-mass-matched healthy controls, COPD showed a 25% decrease in rectus femoris CSA (Seymour et al., 2009). In terms of muscle protein turnover, stable COPD patients have lower levels of muscle-specific atrophy F-box protein in the quadriceps muscle (Lemire et al., 2012; Natanek et al., 2013). Additionally, pectoralis CSA has been found to be significantly related to forced expiratory volume in one second to forced vital capacity ratio (Tashiro et al., 2021), and decreased pectoralis CSA has been associated with increased all-cause mortality, independent of body mass index and primary muscle mass (Mason et al., 2022). Functional changes in COPD muscle include decreased muscle strength and endurance. A meta-analysis of 728 COPD patients and 440 healthy controls demonstrated a significant decrease in quadriceps endurance in COPD patients compared with controls (Evans et al., 2015). Decreases in knee extension absolute strength (Roig et al., 2011), rectus lateralis muscle isometric strength per CSA, maximal voluntary capacity (MVC), and endurance have also been observed in COPD patients (Debigaré et al., 2003; Faucher et al., 2004). In addition, COPD patients exhibited decreased strength and endurance in digital flexors and biceps muscles (Faucher et al., 2004; Franssen et al., 2005). Furthermore, a 2 year study involving 162 moderate-to-severe COPD patients found that only quadriceps MVC and age were predictors of mortality (Swallow et al., 2007), which was supported

by another 4 year study (Spositon et al., 2022). Metabolic changes in COPD patients mainly involve alterations in muscular fiber types, with a decreased proportion of slow muscle fibers. Decreased size, oxidative capacity, and mitochondrial density of type I fibers were observed in the quadriceps of COPD patients (Mathur et al., 2014). Additionally, there was a decrease in the proportion of type I muscle fibers and an increase in type IIB and type IIX muscle fibers in the peripheral muscles of COPD patients (Debigaré et al., 2003; Torres et al., 2011). These changes are accompanied by a decrease in oxidase (citrate synthase and 3-hydroxyacyl-coA dehydrogenase) activity and oxidative capacity, as well as an increase in the glycolytic enzyme phosphofructokinase in COPD's quadriceps muscles (Jakobsson et al., 1995; Mador and Bozkanat, 2001). In conclusion, COPD patients exhibit significant abnormality in skeletal muscle, which are correlated with clinical function and exacerbation outcomes.

2.2 Overview of autophagy

Autophagy, as one of the proteolysis systems of organisms, plays a crucial role in maintaining cellular homeostasis by removing long-lived proteins and damaged organelles (Wang et al., 2018). Various pro-atrophic stimuli, such as starvation, muscle denervation, hypoxia, oxidative stress, organelle damage, and the accumulation of mutant proteins, can induce autophagy (Gouzi et al., 2018). In skeletal muscle dysfunction in COPD, the primary types of autophagy studied are macroautophagy and mitophagy. Macroautophagy degrades cellular components by enclosing them in autophagosomes, which then fuse with lysosomes to form autophagolysosomes (Mizushima et al., 2008). The initiation of macroautophagy is regulated by Unc-51, like autophagy, activating kinase 1 (ULK1), which is inactivated by the mammalian target of rapamycin (mTOR) (Singh et al., 2017). On the other hand, AMP-activated protein kinase (AMPK) inhibits mTOR, thereby activating ULK1 and forkhead box O (FOXO) 3 to promote macroautophagy (Bujak et al., 2015). Following initiation, the autophagic nucleation process is induced by the class III phosphatidylinositol 3-kinase (PI3K III) complex, consisting of Beclin1, vesicle sorting protein 34, and vesicle sorting protein 15. This complex recruits ATG proteins and lipids in the cytoplasm to synthesize a large number of bilayer membrane structures. These membrane structures continue to elongate and eventually encapsulate intracellular material, forming an autophagosome structure under the action of ATG12-ATG5 and ATG8/LC3 conjugation systems (Suzuki et al., 2013). The formation of LC3BII, a marker of autophagosome formation, is positively correlated with the number of autophagosomes (Mizushima and Yoshimori, 2007). Subsequently, the autophagosome, along with numerous autophagy-related proteins (ATG5-ATG12-ATG16-like1 complex), transports ubiquitination-labeled cellular waste to the lysosome and merges with it to form the autophagolysosome. Within the autophagolysosome, the waste is digested and degraded into small molecules such as amino acids, nucleic acids, and fatty acids, which are then recycled back into the cytoplasm (Suzuki et al., 2013; Wang et al., 2018). In this process, p62, which links ubiquitinated substrates with LC3B, is integrated into the autophagolysosome and degraded (Jeong et al., 2019).

Mitophagy is a selective autophagic response that targets specifically damaged mitochondria (Klionsky et al., 2021). The two well-studied mitophagy pathways in mammalian cells are ubiquitination-dependent and receptor-mediated mitophagy. Mitochondrial injury leads to decreased mitochondrial membrane potential, resulting in increased stability of PINK1 on the outer mitochondrial membrane. PINK1 then recruits the E3 ubiquitin ligase PARKIN to the outer mitochondrial membrane. Self-phosphorylation of PINK1 activates PARKIN, which polyubiquitinates mitochondrial proteins. Theses ubiquitinated proteins bind to the structural domains of autophagy receptors (p62, calcium-binding, and coiled-coil domain-containing protein 2 and Optineurin) and form autophagosomes. The autophagosome subsequently fuses with lysosomes, leading to mitochondrial degradation (Fivenson et al., 2017; Lazarou et al., 2015; Su et al., 2023). Receptor-mediated mitophagy occurs through the interaction between LC3B interaction area on the receptor proteins and LC3B or GABA receptor-associated protein on autophagic vesicles. FUN14 domain-containing protein 1 (FUNDC1) and BCL2/adenovirus E1B interacting protein 3 (BNIP3), which have the LC3B interaction area, act as mitophagy receptor protein in the mitochondrial outer membrane (M. Chen et al., 2016). The homolog of BNIP3,

unedited

BNIP3/NIX, can mediate mitophagy through the GABA receptor-associated protein (Romanello and Sandri, 2015; Su et al., 2023). Additionally, BNIP3 can activate macroautophagy by inhibiting mTOR or competitive binding with BCL-2 to release Beclin1 (Bellot et al., 2009; Lin et al., 2014).

3 Role of autophagy in COPD skeletal muscle dysfunction

3.1 Macroautophagy dysregulation mediates COPD skeletal muscle dysfunction

Macroautophagy-related changes in COPD skeletal muscle are related to structural alterations, such as CSA and muscle mass. Studies have indicated that LC3BII, Beclin1, and p62 proteins and autophagosome numbers increase in the vastus lateralis of COPD, with more prominent changes in COPD sarcopenia (Guo et al., 2013; Hussain and Sandri, 2013; Kneppers et al., 2017). Furthermore, LC3B lipidation has found to be negatively correlated with COPD thigh CSA (Guo et al., 2013). The activation levels of FOXO, which inducts the expression of autophagic proteins (e.g., LC3B, GABA receptor-associated protein, BNIP3 and BNIP3L), are negatively correlated with COPD body mass index ($r = -0.96$) (Puig-Vilanova et al., 2015). This seems to indicate that the level of macroautophagy is significantly elevated in COPD skeletal muscle and is significantly negatively correlated with skeletal muscle structure. It is consistent that autophagy flux in the myoblasts of COPD is increased (Gouzi et al., 2018). An in vitro study that applied autophagy inhibitor 3-MA to cultured COPD myotubes found a significant increase in the diameter of myotubes (Gouzi et al., 2018). Therefore, inhibiting skeletal muscle autophagy in COPD led to an improvement in skeletal muscle structure. An interesting finding is that oxidative stress may affect COPD skeletal muscle structure through decreased autophagy. In fact, the administration of ascorbic acid on COPD myotubes decreased the LC3B lipidation ratio and p62 levels, along with a significant increase in myotube CSA (Gouzi et al., 2018).

The dysregulation of macroautophagy has been implicated in the muscle function alteration in COPD. In the vastus lateralis muscle of COPD patients, the levels of p-AMPK and total AMPK are significantly increased (Guo et al., 2013). This increase can activate ULK, leading to autophagy promotion through phosphorylation at Ser317 and Ser777 (J. Kim et al., 2011). COPD patients with muscle wasting have significantly decreased levels of p-ULK1 (Ser757, inhibitory)/ULK1 in the vastus lateralis muscle compared to those without muscle wasting (Kneppers et al., 2017). Animal models of COPD further support the dysregulation of macroaugtophagy, with increased protein levels of ULK1 in the quadriceps muscle (Mao et al., 2020), and decreased levels of p-ULK1 (Ser757)/ULK1 in the gastrocnemius muscle (Ceelen et al., 2017). The elevation of AMPK and ULK1 may contribute to the deterioration of muscle function in skeletal muscle. Although direct evidence supporting these findings in COPD is lacking, similar changes have been observed in other disease models. For instance, Laker et al. (2017) demonstrated the necessary role of AMPKα in the activation of autophagy after endurance exercise. The deletion of ULK1 hindered the improvement of glucose tolerance after exercise. In COPD skeletal muscle, discrepant findings have been reported. Gouzi et al. (2018) found that the use of autophagy inhibitors improved COPD muscle fiber diameter, while Balnis et al. (2022) found a decrease in autophagosome formation rate in COPD satellite cells and a decrease in myogenesis, which is significantly alleviated by the systemic administration of spermidine (an autophagy inducer). These differences may arise from the different functions of muscle fibers and satellite cells. Muscle fibers generate force and movement, while satellite cells are responsible for regenerating and repairing damaged muscle. Therefore, they may have distinct needs and responses to autophagy regulation. Similarly, previous studies have demonstrated that both activation and inhibition of autophagy can improve lipid metabolism and glucose uptake in obese mice (He et al., 2012; K. H. Kim et al., 2013).

Overall, it is evident that the expression levels of macroautophagy-related proteins are significantly increased in COPD skeletal muscle, and they are negatively correlated with muscle function and structure. Yet, the role of autophagy in different muscle cells in COPD skeletal muscle function is inconsistent.

3.2 Acceleration of mitophagy improves COPD skeletal muscle dysfunction

Unedited

Promoting mitophagy increases COPD myotube CSA and myosin heavy chain (MHC) and decreases E3 ubiquitination ligase. BNIP3 mRNA in the vastus lateralis of COPD patients increases significantly (Guo et al., 2013). In COPD animal models, BNIP3 proteins increase in the soleus and tibialis anterior muscle and BNIP3 mRNA increase in gastrocnemius (Balnis et al., 2022; Ceelen et al., 2017; Mano et al., 2022). In vivo, cigarette smoke exposure decreases the MHC of gastrocnemius muscle in PARKIN$^{-/-}$ mice compared with wild-type controls (Ito et al., 2022). Additionally, cigarette smoke extract-treated C2C12 cells with PARKIN siRNA showed myotube atrophy, and PARKIN overexpression further confirmed these results. These findings suggest that the increase in mitophagy-related proteins may be a protective response, as mitophagy helps to remove impaired mitochondria and prevent their accumulation. Impaired mitochondria can lead to increased mitochondrial permeability transition pore opening, the activation of the inner membrane anion channel, and the leakage of more reactive oxygen species (ROS) (Zorov et al., 2014). Mitochondria-derived ROS is known to contribute to oxidative damage and skeletal muscle dysfunction in COPD skeletal muscle (Puente-Maestu et al., 2009; Puente-Maestu et al., 2012). Promoting mitophagy can accelerate the removal of mitochondria-derived ROS, thus alleviating skeletal muscle dysfunction (Ito et al., 2022). The accumulation of damaged mitochondria and increased 4-hydroxynonenal (4-HNE) was observed in PARKIN$^{-/-}$ mice, while the administration of mitochondrial antioxidant significantly improved the decreased MHC and diameter in cigarette smoke extract-treated C2C12 myotubes (Ito et al., 2022). Additionally, astaxanthin was shown to decrease 4-HNE, BNIP3, and atrophy F-box protein in soleus, leading to increased muscle mass, type I muscle fiber ratio, and CSA of type I and IIA (Mano et al., 2022).

Mitophagy dysfunction has been linked to skeletal muscle strength and overall exercise capacity in COPD, with BNIP3 proteins in quadriceps being negatively correlated with the percentage of the 6-minute walking distance to predicted value ($r = -0.736$) (Gouzi et al., 2018), and PARKIN$^{-/-}$ COPD mice exhibiting a weaker grip strength compared to wild-type COPD mice (Ito et al., 2022). These findings support the role of mitophagy in COPD skeletal muscle structure, and suggest that it plays a protective role in removing impaired mitochondria.

According to the above studies, the level of macroautophagy-related proteins is significantly increased in COPD skeletal muscle, and the effect of regulating macroautophagy may be cell type-dependent. Insufficient mitophagy can lead to structural and functional dysfunction of skeletal muscle in COPD. For a detailed summary of the relevant literature, please refer to Table 1.

unedited

Table 1 Studies on skeletal muscle autophagy changes in COPD in the past 5 years

Studies	Groups	Changed major autophagy indicators	Changed muscle dysfunction indicators
Leermakers et al., 2018	EG: Mild-to-severe COPD; CG: Healthy controls	**Protein level** of BNIP3L↑, FUNDC1↓, PARKIN↑, LC3BI↑, LC3BII/LC3BI↓, p62→ in VL **mRNA level** of PARKIN2↑, OPTN↑	Myh7 mRNA↓
Gouzi et al., 2018	EG: muscle cells form VL of moderate-to-severe COPD; CG: muscle cells form VL of sedentary healthy subjects	**Myoblast** protein level of LC3↑, LC3BII/LC3BI↑, and p62↑; mRNA expression of p62↑, BNIP3↑; autophagic flux↑ **Myotube** protein level of LC3↑, p62↑; mRNA expression of p62↑, BNIP3↑; autophagic flux	
	EG: VL cells of COPD treated with 3-MA CG: VL cells of COPD control	Myotube protein level of p62↓, BNIP3↓	Myotube diameter↑
	EG: COPD VL myotube treated with ascorbic acid CG: COPD VL myotube control	Myotube protein level of p62↓, BNIP3↓, LC3BII/LC3BI↓	Myotube diameter↑
Leermakers et al., 2020	EG: male mice + lipopolysaccharide CG: male mice + saline	**Protein level** of BNIP3↑, BNIP3L-II↑, FUNDC1↓, PARKIN2↑, LC3BII↑ in GN after injection for 96 h. **mRNA level** of BNIP3L↑, BNIP3↑, LC3B↑, GABARAPL1↑, p62↑in GN after injection for 48 h.	GN weight/body weight↓
Mao et al., 2020	EG: Rats + CSE + klebsiella pneumonia suspension + saline CG: Rats + saline	**Protein level** of LC3BII/LC3BI↑, ULK1↑, PINK↑, PARKIN↑, AMPKα↓, p-AMPKα↓; **mRNA level** of LC3B↑, ULK1↑, PINK1↑, PARKIN↑, AMPKα↓ in quadriceps	
	L6 + CSE L6 control	**Protein level** of ULK1↑, PINK↑, PARKIN↑, AMPKα↓, p-AMPKα↓, mTOR↓; **mRNA level** of AMPKα↓, ULK1↑, PINK1↑, PARKIN↑, mTOR↓ in quadriceps	
Balnis et al., 2022	EG: *IL13*^{TG} COPD mice CG: WT mice	**mRNA of** ULK1↑, Beclin1↑, BNIP3↑, LC3↑, p62↑, ATG7↑, ATG3↑ in TA	Grip strength↓, EDL maximum absolute isolated contraction↓, EDL CSA↓, GN muscle mass↓, TA muscle mass↓,

unedited

Table 1 Studies on skeletal muscle autophagy changes in COPD in the past 5 years (continued)

Studies	Groups	Changed major autophagy indicators	Changed muscle dysfunction indicators
	EG: *IL13*^{TG} COPD mice with acute TA injury CG: WT mice with acute TA injury		CSA at Day 14 after injury↓
	EG: TA satellite cells from *IL13*^{TG} or *MMP1*^{TG} COPD mice CG: TA satellite cells from WT mice	**mRNA of** Beclin1↑	Cell proliferation↓, cell counts→; mRNA of MyoD↓, Myf5↓, eMHC↓
	Group1: bafilomycin treated GFP-LC3+ *IL13*^{TG} mice Group2: GFP-LC3+ *IL13*^{TG} mice CG: GFP-LC3+ WT mice	**Protein level of** LC3↑ (Group2 compared with CG; Group2 compared with Group1) in muscle satellite cells	
	EG: GFP-LC3+ *IL13*^{TG} mice CG: GFP-LC3+ WT mice	**Protein level of** LC3↑	
Ito et al., 2022	**C2C12 were divided into:** Control group CSE exposure PARKIN siRNA + CSE PARKIN siRNA PARKIN overexpression + CSE PARKIN overexpression	**CSE + WT compared with CG:** Protein of PARKIN↓ mRNA of PARKIN↓ **CSE + PARKIN siRNA compared with CSE + WT:** Protein of p62↑, PINK1↑	**CSE + WT compared with CG:** Protein of MHC↓, average myotube diameters↓, MuRF1↑ **CSE + PARKIN siRNA compared with CSE + WT:** MHC→, average myotube diameters↓, MuRF1↑ **CSE + PARKIN overexpression compared with CSE + WT:**
	Female WT C57BL/6J mice + CSE Female WT C57BL/6J mice control Female PARKIN KO mice + CSE Female PARKIN KO mice control	**CSE +WT compared with WT:** Protein level of PARKIN↓, p62→, PINK1→ in GN **PARKIN KO + CSE compared with WT + CSE:** Protein level of PARKIN↓, p62↑, PINK1↑in GNs	**CSE +WT compared with WT:** Grip force↓, GN muscle weight/length→, TA muscle weight/length→, EDL muscle weight/length→, SOL muscle weight/length↓, GN average muscle fiber diameter↓ **PARKIN KO + CSE compared with WT + CSE:** muscle weight/length↓, GN average muscle fiber diameter↓, MHC↓
	EG: COPD patients with sarcopenia CG: Patients with no COPD	Protein level of PARKIN↓, PINK1↑, p62↑ in gluteus maximus muscle	MuRF1↑ in gluteus maximus muscle

unedited

Table 1 Studies on skeletal muscle autophagy changes in COPD in the past 5 years (continued)

Studies	Groups	Changed major autophagy indicators	Changed muscle dysfunction indicators
Mano et al., 2022	**Male C57BL/6 J mice divided into:** EG: PPE treated CG: saline treated	Soleus protein of BNIP3↑	Weight of the soleus↓, %type I muscle↓, %type IIA↑ and CSA↓, MuRF1↑ and Atrogin1↑ protein in the soleus;
	EG: PPE+ astaxanthin treated CG: PPE treated	BNIP3 protein↓	Atrogin1↓, MuRF1→, soleus weight↑, %type I fibers↑, type I CSA↑, type IIA CSA↑
	EG: PPE+p38 MAPK inhibitor treated CG: PPE treated	BNIP3 protein↓	MuRF1↓, soleus weight↑, %type I fibers↑, type I CSA↑, type IIA CSA↑

↑: increased compared with CG unless noted; ↓: decreased compared with CG unless noted; →: has no changes compared with CG unless noted; AMPK: AMP-activated protein kinase; ATG: autophagy; Beclin1: Bcl2 interacting protein 1; Atrogin1: muscle atrophy F-box; BNIP3: bcl 2 adenovirus E1B 1interacting protein 3; CG: control group; COPD: chronic obstructive pulmonary disease; CSA: cross-sectional aera; EG: experimental group; CSE: cig-arette smoke extract; EDL: extensor digitorum longus; eMHC: embryonic myosin heavy chain; FUNDC1: FUN14 domain-containing protein 1; GABARAPL1: γ-aminobutiric acid receptor-associated protein-like 1; GN: gastrocnemius muscle; LC3: microtubule-associated protein light chain 3; mTOR: mammalian target of rapamycin; MuRF1: muscle ring finger protein l; Myf5: myogenic factor 5; Myh7: recombinant myosin heavy chain 7; MyoD1: myogenic differen-tiation 1; PINK1: PTEN induced kinase 1; PPE: porcine pancreatic elastase; TA: tibialis anterior muscle; ULK: Unc-51-like kinase 1; VL: vastus lateralis muscle; 3-MA: 3-Methyladenine

Unedited

4 Mechanisms of autophagy leading to COPD skeletal muscle dysfunction

4.1 Macroautophagy regulates the function of muscle satellite cells in COPD

MuSCs are skeletal muscle resident stem cells located below the basal lamina of myofibers, which are quiescent in the general state and can proliferate and differentiate into myofibers in response to stimuli such as muscle injury (Henrot et al., 2023). MuSCs play an essential role in myoblast nuclear turnover and muscle regeneration. Autophagy regulates the maintenance of the quiescence, activation, proliferation, and differentiation of MuSCs (W. Chen et al., 2022). Quiescent MuSCs maintain the renewal of organelles and proteins through autophagy. Autophagy is induced during the activation and proliferation of MuSCs after injury, which may result from high energy demand. Autophagy is activated again during muscle differentiation (W. Chen et al., 2022).

The dysfunction of MuSCs in COPD has been investigated. In COPD patients, MuSCs have delayed activation, reduced myosin heavy chain expression during myotube formation, and decreased myotube diameter when cultured in vitro (Pomiès et al., 2015; Thériault et al., 2014). The reduced proliferative capacity of MuSCs can also be detected in COPD (Balnis et al., 2022). Recent studies have revealed significant changes in autophagy levels in COPD MuSCs. Balnis et al. (2022) found dysregulated autophagy in COPD MuSCs by RNAseq analysis and Western blot validation. Bafilomycin (autophagy inhibitor) utilization further demonstrated that the autophagosome turnover efficiency of MuSCs was reduced. The whole-body application of spermidine, a natural inducer of autophagy (3 mM drinking water for 4 weeks), significantly increased the proliferation rate and myogenesis of MuSCs after injury. The study suggests reduced autophagy in COPD MuSCs may contribute to muscle regenerative dysfunction. Spermidine, which the study used, is a natural inducer of autophagy (Eisenberg et al., 2009). One study found that spermidine could not preserve the proliferative ability of MuSCs in ATG7 (essential for canonical macroautophagy) knocked out mice, suggesting that autophagy mediates the pro-proliferative ability of spermidine on MuSCs (García-Prat et al., 2016). Mechanistically, Zhang et al. (2018) found that, after the spermidine treatment of MuSCs, the affinity of Smad3 for the promoters of the myogenic factors Myf5 and MyoD was decreased, and the myogenic capacity was reduced. The above studies indicate that the activation of COPD autophagy can restore the regenerative capacity of MuSCs.

unedited

4.2 Mitophagy sustains mitochondrial homeostasis in COPD muscle

Mitochondria play a central role in cellular energy homeostasis, and regulate various processes such as amino acid synthesis, lipid metabolism, and apoptotic signaling (Eldeeb et al., 2022). Autophagy helps decrease mitochondrial oxidative damage and maintain mitochondria function. Inhibiting ATG7 in myoblasts leads to decreased mitochondrial membrane potential, mitochondria function-related proteins (peroxisome proliferative activated receptor γ coactivator 1 α, mitochondrial dynamin like GTPase and dynamin-related protein 1), and increased 4-HNE (Baechler et al., 2019).

In COPD, mitochondrial dysfunction in skeletal muscle is evident through reduced mitochondrial density, dysregulated respiratory function, and the increased production of mitochondrial ROS (Gifford et al., 2018). The main consequence of mitochondrial dysfunction is a decrease in muscle oxidative capacity, resulting in increased lactate production during low-intensity and endurance exercise (Puente-Maestu et al., 2013). The reduction in mitochondrial content in quadriceps muscle fibers results in decreased oxidative capacity, which is consistent with a reduction in type I muscle fibers (Puente-Maestu et al., 2013). Correcting skeletal muscle mitochondrial dysfunction significantly improves COPD skeletal muscle function, as seen by reduced atrophy and increased MHC levels after administering mitochondrial ROS-specific antioxidants to COPD myotubes (Ito et al., 2022). Overall, mitochondrial dysfunction mediates the dysfunction observed in COPD skeletal muscle.

The changes in mitophagy status are significantly correlated with mitochondrial function in COPD skeletal

muscle. The PARKIN$^{-/-}$ COPD mice also exhibited increased translocase of the outer membrane 20, 4-HNE, and damaged mitochondrial accumulation. Meanwhile, MHC in the gastrocnemius and average CSA decrease significantly (Ito et al., 2022). Impaired mitophagy, swollen mitochondrial cristae, and increased mitochondrial membrane disruption were observed in CSE-induced C2C12 myotubes after PARKIN siRNA transduction, accompanying increased MuRF1 and decreased myotube (Ito et al., 2022). Although the role of receptor protein-mediated mitochondrial autophagy in COPD skeletal muscle function has not been investigated, studies have shown a significant increase in BNIP3 protein levels in COPD skeletal muscle (Balnis et al., 2022; Guo et al., 2013; Mano et al., 2022). BNIP3 is known to decrease oxidative phosphorylation, increase mitochondrial protease activity, and potentially promote mitochondrial homeostasis (Rikka et al., 2011). Furthermore, FUNDC1 in COPD skeletal muscle decreases. These studies may elucidate that FUNDC1 and BNIP3 are regulated by different mechanisms, although both of them mediate receptor-dependent mitophagy. Overall, the available evidence suggests that mitochondrial autophagy may preserve skeletal muscle function in COPD by clearing damaged mitochondrial ROS and maintaining mitochondrial integrity.

The mechanism of autophagy leading to COPD skeletal muscle dysfunction is depicted in Figure 1.

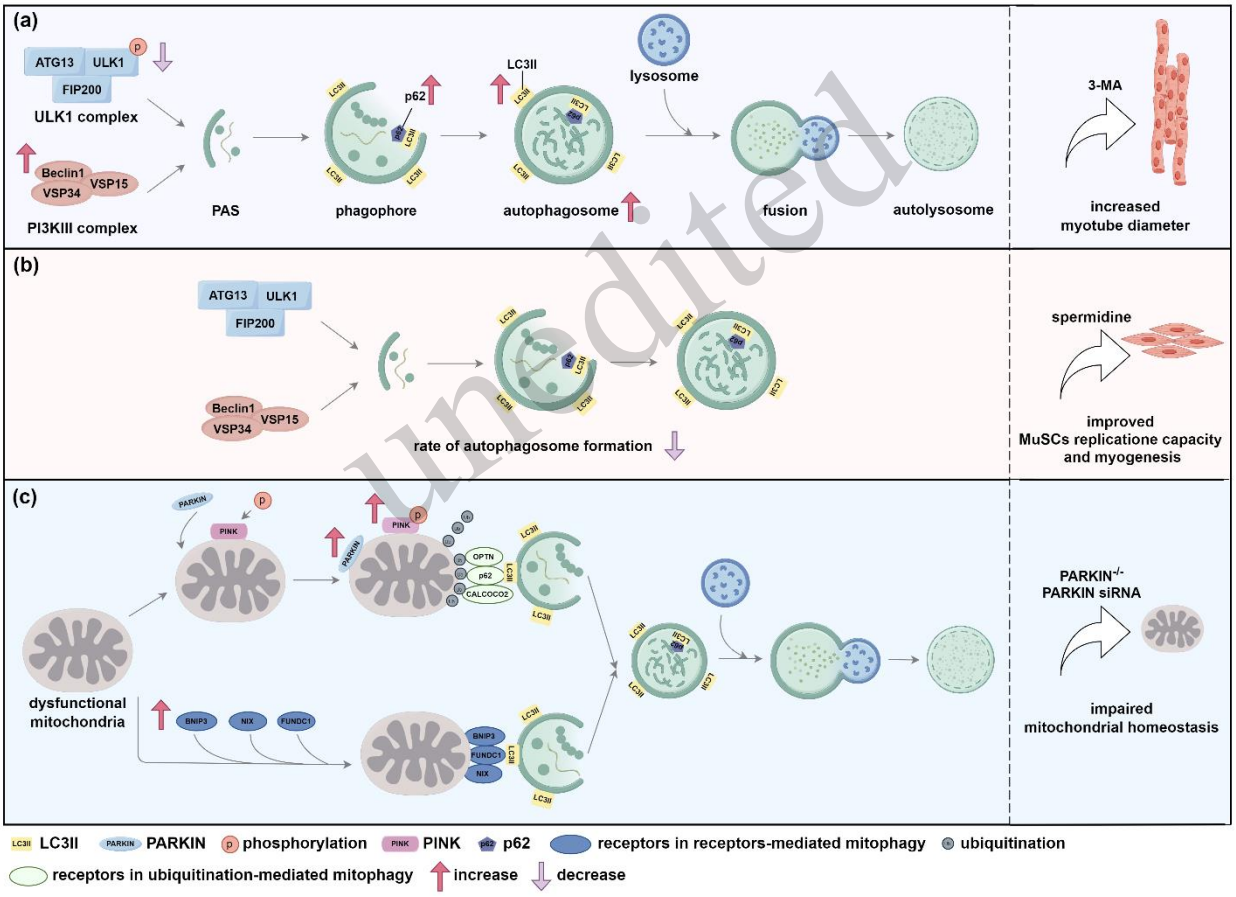

Figure 1. Mechanism of autophagy leading to COPD skeletal muscle dysfunction. (a) In COPD skeletal muscle, myotubes and myoblasts, autophagy-related proteins were found to be significantly increased. The use of 3-MA (autophagy inhibitor) in COPD myotubes led to an increased myotube diameter. (b) However, in MuSCs in COPD, the rate of autophagosome formation decreased and spermidine (autophagy inducer) helped increase diameters of MuSCs. (c) Mitophagy-related proteins were observed to increase, and PARKIN underexpression may promote muscle dysfunction through exacerbating mitochondrial homeostasis. BNIP3: BCL2/adenovirus E1B interacting protein 3; CALCOCO2: calcium-binding and coiled-coil domain-containing protein 2; COPD: chronic obstructive pulmonary disease; FIP200: 200kD FAK family kinase-interacting protein; FUNDC1: FUN14 domain-containing protein 1; Beclin1: myosin-like BCL2 interacting protein; LC3II: microtubule-associated protein light chain 3 II; MuSCs: muscle satellite cells; NIX: BCL2/adenovirus E1B interacting protein

unedited

3-like; OPTN: Optineurin; PAS: pre-autophagosomal structure; ULK1: Unc-51 like autophagy activating kinase 1; VSP: vesicle sorting protein: 3-MA: 3-methyladenine. The figure is generated by Figdraw (figdraw.com).

5 Conclusion and perspectives

5.1 Conclusion

In COPD skeletal muscle, autophagy-related proteins were significantly increased. Mitophagy may help maintain COPD skeletal muscle homeostasis, while macroautophagy showed opposite regulatory effects in myotubes and muscle satellite cells. Mechanistically, macroautophagy can regulate the myogenesis and regenerative capacity of satellite cells, while mitophagy helps restore mitochondrial homeostasis in skeletal muscle cells. Studying the effect and mechanism of autophagy on COPD skeletal muscle dysfunction is helpful in gaining a new perspective on understanding this issue and providing a theoretical basis for its clinical treatment.

5.2 Perspectives

This is an updated review on the role of autophagy in COPD skeletal muscle dysfunction. Numerous studies have been published in the ten years since the last review (Hussain and Sandri, 2013). Unfortunately, there are few articles (Balnis et al., 2022; Gouzi et al., 2018; Ito et al., 2022) on regulating the level of autophagy and observing the changes of skeletal muscle structure and function in COPD. Moreover, the results from these few articles are inconsistent. Based on the current research, there are several limitations and areas for improvement that need to be addressed in future studies. Firstly, most studies have only examined the expression of autophagy-related molecular markers at the protein and mRNA levels, without dynamically monitoring autophagic flux. Consequently, these studies are not sufficient to effectively reflect the true changes in autophagy and require a more cautious interpretation of the findings. To address this limitation, the use of GFP-LC3 mice can allow for the dynamic observation of changes in autophagy in COPD muscle cells after drug treatment (Balnis et al., 2022). Furthermore, changes in autophagy can be inferred by observing changes in autophagy-related protein expression and using autophagy modulators such as bafilomycin or 3-MA to measure autophagic flux. These dynamic detection methods can better reflect the true state of the disease. Additionally, in exploring changes in autophagy, further validation of changes in different stages of autophagy, such as initiation, nucleation, elongation, maturation, fusion, and degradation, is needed to achieve the precise regulation of autophagy. Secondly, the detection of autophagy levels in COPD skeletal muscle may be influenced by various factors, such as disease stage, muscle fiber type, and muscle cell differentiation status. Many studies have demonstrated that autophagy plays different roles at different stages of disease and physiological processes (Fiacco et al., 2016). It is crucial to understand whether there is differential regulation of autophagy in COPD skeletal muscle at different disease stages for the precise regulation of autophagy to improve COPD skeletal muscle function. For instance, Leermakers et al. (2020) found that the protein levels of BNIP3 and BNIP3L-II in the gastrocnemius muscle of mice reached a peak at 48 hours after the induction of a pneumonia model, while BNIP3L reached its lowest abundance at 72 hours. In soleus, there was no significant difference in the protein and mRNA levels of BNIP3L. Consistently, chronic ATG7 knockout mice showed only increased glycolytic muscle apoptosis protein and protein hydrolytic enzyme activity, with decreased strength and twitch kinetics (Paré et al., 2017). Furthermore, different types of muscle fibers have different responses to changes in autophagy, highlighting the need for the further exploration of the differential regulation of autophagy in different fiber types of COPD. Additionally, autophagy plays different roles in different statuses of muscle satellite cells, yet there is no research on the role of autophagy in different stages of muscle satellite cells in COPD. Furthermore, autophagy is a promising target for the targeted therapy of COPD skeletal muscle dysfunction. The systemic application of autophagy activators has shown improvement in COPD skeletal muscle dysfunction in rodent models. However, it is important to consider the potential impact of systemic

unedited

autophagy intervention on autophagy in other organs that could affect COPD skeletal muscle. Moreover, due to the multiple biological effects of autophagy modulators, the interpretation of experimental results is limited. To overcome these limitations, the development of skeletal muscle-specific autophagy regulators and precise autophagy modulation drugs would help to further elucidate the role and specific mechanisms of autophagy in skeletal muscle dysfunction in COPD patients.

Acknowledgments

This work is supported by the National Natural Science Foundation of China (grant number 82172551), the Health Discipline Leader Project of Shanghai Municipal Health Commission (grant number 2022XD044).

Author contributions

Conceptualization and design: Weibing WU, Xiaodan Liu; Drafting: Xiaoyu Han; Analysis and interpretation: Xiaoyu Han, Peijun Li, Meiling Jiang; Critical revising: Yuanyuan Cao, Yingqi Wang, Linhong Jiang, Peijun Li, Meiling Jiang, Xiaoyu Han and Weibing Wu. All authors have read and agreed the final manuscript.

Compliance with ethics guidelines

Xiaoyu HAN, Peijun LI, Meiling JIANG, Yuanyuan CAO, Yingqi WANG, Linhong JIANG, Xiaodan LIU, Weibing WU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

Unedited

References

Baechler BL, Bloemberg D, Quadrilatero J, 2019. Mitophagy regulates mitochondrial network signaling, oxidative stress, and apoptosis during myoblast differentiation. *Autophagy*, 15(9):1606-1619. https://doi.org/10.1080/15548627.2019.1591672

Balnis J, Drake LA, Singer DV, *et al*., 2022. Deaccelerated myogenesis and autophagy in genetically induced pulmonary emphysema. *Am J Respir Cell Mol Biol*, 66(6):623-637. https://doi.org/10.1165/rcmb.2021-0351OC

Bellot G, Garcia-Medina R, Gounon P, *et al*., 2009. Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of bnip3 and bnip3l via their bh3 domains. *Mol Cell Biol*, 29(10):2570-2581. https://doi.org/10.1128/mcb.00166-09

Benz E, Trajanoska K, Lahousse L, *et al*., 2019. Sarcopenia in copd: A systematic review and meta-analysis. *Eur Respir Rev*, 28(154):190049. https://doi.org/10.1183/16000617.0049-2019

Bernard S, Leblanc P, Whittom F, *et al*., 1998. Peripheral muscle weakness in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*, 158(2):629-634. https://doi.org/10.1164/ajrccm.158.2.9711023

Bujak AL, Crane JD, Lally JS, et al., 2015. Ampk activation of muscle autophagy prevents fasting-induced hypoglycemia and myopathy during aging. *Cell Metab*, 21(6):883-890. https://doi.org/10.1016/j.cmet.2015.05.016

Ceelen JJM, Schols A, Van Hoof SJ, *et al*., 2017. Differential regulation of muscle protein turnover in response to emphysema and acute pulmonary inflammation. *Respir Res*, 18(1):75. https://doi.org/10.1186/s12931-017-0531-z

Chen M, Chen Z, Wang Y, *et al*., 2016. Mitophagy receptor fundc1 regulates mitochondrial dynamics and mitophagy. *Autophagy*, 12(4):689-702. https://doi.org/10.1080/15548627.2016.1151580

Chen W, Chen Y, Liu Y, *et al*., 2022. Autophagy in muscle regeneration: Potential therapies for myopathies. *J Cachexia Sarcopenia Muscle*, 13(3):1673-1685. https://doi.org/10.1002/jcsm.13000

Christenson SA, Smith BM, Bafadhel M, *et al*., 2022. Chronic obstructive pulmonary disease. *Lancet*, 399(10342):2227-2242. https://doi.org/10.1016/s0140-6736(22)00470-6

Debigaré R, Côte CH, Hould FS, *et al*., 2003. In vitro and in vivo contractile properties of the vastus lateralis muscle in males with copd. *Eur Respir J*, 21(2):273-278. https://doi.org/10.1183/09031936.03.00036503

Eisenberg T, Knauer H, Schauer A, *et al*., 2009. Induction of autophagy by spermidine promotes longevity. *Nat Cell Biol*, 11(11):1305-1314. https://doi.org/10.1038/ncb1975

Eldeeb MA, Thomas RA, Ragheb MA, *et al*., 2022. Mitochondrial quality control in health and in parkinson's disease. *Physiol Rev*, 102(4):1721-1755. https://doi.org/10.1152/physrev.00041.2021

Evans RA, Kaplovitch E, Beauchamp MK, *et al*., 2015. Is quadriceps endurance reduced in copd?: A systematic review. *Chest*, 147(3):673-684. https://doi.org/10.1378/chest.14-1079

Faucher M, Steinberg JG, Barbier D, *et al*., 2004. Influence of chronic hypoxemia on peripheral muscle function and oxidative stress in humans. *Clin Physiol Funct Imaging*, 24(2):75-84. https://doi.org/10.1111/j.1475-097X.2004.00533.x

Fermont J M, Masconi K L, Jensen M T, *et al*.,2019 Biomarkers and clinical outcomes in COPD: a systematic review and meta-analysis. *Thorax*, 74(5): 439-446. http://doi.org/10.1136/thoraxjnl-2018-211855

Fiacco E, Castagnetti F, Bianconi V, *et al*., 2016. Autophagy regulates satellite cell ability to regenerate normal and dystrophic muscles. *Cell Death Differ*, 23(11):1839-1849. https://doi.org/10.1038/cdd.2016.70

Fivenson EM, Lautrup S, Sun N, *et al*., 2017. Mitophagy in neurodegeneration and aging. *Neurochem Int*, 109:202-209.

https://doi.org/10.1016/j.neuint.2017.02.007
Franssen FM, Broekhuizen R, Janssen PP, *et al*., 2005. Limb muscle dysfunction in copd: Effects of muscle wasting and exercise training. *Med Sci Sports Exerc*, 37(1):2-9. https://doi.org/10.1249/01.mss.0000150082.59155.4f
García-Prat L, Martínez-Vicente M, Perdiguero E, *et al*., 2016. Autophagy maintains stemness by preventing senescence. *Nature*, 529(7584):37-42. https://doi.org/10.1038/nature16187
Gifford JR, Trinity JD, Kwon OS, *et al*., 2018. Altered skeletal muscle mitochondrial phenotype in copd: Disease vs. Disuse. *J Appl Physiol (1985)*, 124(4):1045-1053. https://doi.org/10.1152/japplphysiol.00788.2017
Global Initiatives for Chronic Obstructive Lung Disease. 2023. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease (2023 report). Available from https://goldcopd.org/2023-gold-report-2/ [Accessed on May. 04, 2023].
Gouzi F, Blaquière M, Catteau M, *et al*., 2018. Oxidative stress regulates autophagy in cultured muscle cells of patients with chronic obstructive pulmonary disease. *J Cell Physiol*, 233(12):9629-9639. https://doi.org/10.1002/jcp.26868
Guo Y, Gosker HR, Schols AM, *et al*., 2013. Autophagy in locomotor muscles of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*, 188(11):1313-1320. https://doi.org/10.1164/rccm.201304-0732OC
He C, Bassik MC, Moresi V, *et al*., 2012. Exercise-induced bcl2-regulated autophagy is required for muscle glucose homeostasis. *Nature*, 481(7382):511-515. https://doi.org/10.1038/nature10758
Henrot P, Blervaque L, Dupin I, *et al*., 2023. Cellular interplay in skeletal muscle regeneration and wasting: Insights from animal models. *J Cachexia Sarcopenia Muscle*, 14(2):745-757. https://doi.org/10.1002/jcsm.13103
Hussain SN, Sandri M, 2013. Role of autophagy in copd skeletal muscle dysfunction. *J Appl Physiol (1985)*, 114(9):1273-1281. https://doi.org/10.1152/japplphysiol.00893.2012
Ito A, Hashimoto M, Tanihata J, *et al*., 2022. Involvement of parkin-mediated mitophagy in the pathogenesis of chronic obstructive pulmonary disease-related sarcopenia. *J Cachexia Sarcopenia Muscle*, 13(3):1864-1882. https://doi.org/10.1002/jcsm.12988
Jaitovich A, Barreiro E, 2018. Skeletal muscle dysfunction in chronic obstructive pulmonary disease. What we know and can do for our patients. *Am J Respir Crit Care Med*, 198(2):175-186. https://doi.org/10.1164/rccm.201710-2140CI
Jakobsson P, Jorfeldt L, Henriksson J, 1995. Metabolic enzyme activity in the quadriceps femoris muscle in patients with severe chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*, 151(2 Pt 1):374-377. https://doi.org/10.1164/ajrccm.151.2.7842194
Jeong SJ, Zhang X, Rodriguez-Velez A, *et al*., 2019. P62/sqstm1 and selective autophagy in cardiometabolic diseases. *Antioxid Redox Signal*, 31(6):458-471. https://doi.org/10.1089/ars.2018.7649
Kim J, Kundu M, Viollet B, *et al*., 2011. Ampk and mtor regulate autophagy through direct phosphorylation of ulk1. *Nat Cell Biol*, 13(2):132-141. https://doi.org/10.1038/ncb2152
Kim KH, Jeong YT, Oh H, *et al*., 2013. Autophagy deficiency leads to protection from obesity and insulin resistance by inducing fgf21 as a mitokine. *Nat Med*, 19(1):83-92. https://doi.org/10.1038/nm.3014
Klionsky DJ, Abdel-Aziz AK, Abdelfatah S, *et al*., 2021. Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition)(1). *Autophagy*, 17(1):1-382. https://doi.org/10.1080/15548627.2020.1797280
Kneppers AEM, Langen RCJ, Gosker HR, *et al*., 2017. Increased myogenic and protein turnover signaling in skeletal muscle of chronic obstructive pulmonary disease patients with sarcopenia. *J Am Med Dir Assoc*, 18(7):637.e1-637.e11. https://doi.org/10.1016/j.jamda.2017.04.016
Laker RC, Drake JC, Wilson RJ, *et al*., 2017. Ampk phosphorylation of ulk1 is required for targeting of mitochondria to lysosomes in exercise-induced mitophagy. *Nat Commun*, 8(1):548. https://doi.org/10.1038/s41467-017-00520-9
Lazarou M, Sliter DA, Kane LA, *et al*., 2015. The ubiquitin kinase pink1 recruits autophagy receptors to induce mitophagy. *Nature*, 524(7565):309-314. https://doi.org/10.1038/nature14893
Leermakers, PA, Schols AMWJ, Kneppers AEM, *et al*., 2018. Molecular signalling towards mitochondrial breakdown is enhanced in skeletal muscle of patients with chronic obstructive pulmonary disease (COPD). *Sci Rep*, 8(1):15007. http//doi.org/10.1038/s41598-018-33471-2
Leermakers PA, Remels AHV, Langen RCJ, *et al*., 2020. Pulmonary inflammation-induced alterations in key regulators of mitophagy and mitochondrial biogenesis in murine skeletal muscle. *BMC Pulm Med*, 20(1):20. https://doi.org/10.1186/s12890-020-1047-8
Lemire BB, Debigaré R, Dubé A, *et al*., 2012. Mapk signaling in the quadriceps of patients with chronic obstructive pulmonary disease. *J Appl Physiol (1985)*, 113(1):159-166. https://doi.org/10.1152/japplphysiol.01518.2011
Lin A, Yao J, Zhuang L, *et al*., 2014. The foxo-bnip3 axis exerts a unique regulation of mtorc1 and cell survival under energy stress. *Oncogene*, 33(24):3183-3194. https://doi.org/10.1038/onc.2013.273
Mador MJ, Bozkanat E, 2001. Skeletal muscle dysfunction in chronic obstructive pulmonary disease. *Respir Res*, 2(4):216-224. https://doi.org/10.1186/rr60
Maltais F, Decramer M, Casaburi R, *et al*., 2014a. An official american thoracic society/european respiratory society statement: Update on limb muscle dysfunction in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*, 189(9):e15-62. https://doi.org/10.1164/rccm.201402-0373ST
Maltais F, Decramer M, Casaburi R, *et al*., 2014b. An official american thoracic society/european respiratory society statement: Update on limb muscle dysfunction in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*, 189(9):e15-e62. https://doi.org/10.1164/rccm.201402-0373ST
Mano Y, Tsukamoto M, Wang KY, *et al*., 2022. Oxidative stress causes muscle structural alterations via p38 mapk signaling in

unedited

copd mouse model. *J Bone Miner Metab*, 40(6):927-939. https://doi.org/10.1007/s00774-022-01371-1

Mao J, Li Y, Feng S, *et al*., 2020. Bufei jianpi formula improves mitochondrial function and suppresses mitophagy in skeletal muscle via the adenosine monophosphate-activated protein kinase pathway in chronic obstructive pulmonary disease. *Front Pharmacol*, 11:587176. https://doi.org/10.3389/fphar.2020.587176

Mason SE, Moreta-Martinez R, Labaki WW, *et al*., 2022. Longitudinal association between muscle loss and mortality in ever smokers. *Chest*, 161(4):960-970. https://doi.org/10.1016/j.chest.2021.10.047

Mathur S, Brooks D, Carvalho CR, 2014. Structural alterations of skeletal muscle in copd. *Front Physiol*, 5:104. https://doi.org/10.3389/fphys.2014.00104

Mizushima N, Yoshimori T, 2007. How to interpret lc3 immunoblotting. *Autophagy*, 3(6):542-545. https://doi.org/10.4161/auto.4600

Mizushima N, Levine B, Cuervo AM, *et al*., 2008. Autophagy fights disease through cellular self-digestion. *Nature*, 451(7182):1069-1075. https://doi.org/10.1038/nature06639

Natanek SA, Riddoch-Contreras J, Marsh GS, *et al*., 2013. Murf-1 and atrogin-1 protein expression and quadriceps fiber size and muscle mass in stable patients with copd. *Copd*, 10(5):618-624. https://doi.org/10.3109/15412555.2013.781577

Paré MF, Baechler BL, Fajardo VA, *et al*., 2017. Effect of acute and chronic autophagy deficiency on skeletal muscle apoptotic signaling, morphology, and function. *Biochim Biophys Acta Mol Cell Res*, 1864(4):708-718. https://doi.org/10.1016/j.bbamcr.2016.12.015

Pomiès P, Rodriguez J, Blaquière M, *et al*., 2015. Reduced myotube diameter, atrophic signalling and elevated oxidative stress in cultured satellite cells from copd patients. *J Cell Mol Med*, 19(1):175-186. https://doi.org/10.1111/jcmm.12390

Puente-Maestu L, Pérez-Parra J, Godoy R, *et al*., 2009. Abnormal mitochondrial function in locomotor and respiratory muscles of copd patients. *Eur Respir J*, 33(5):1045-1052. https://doi.org/10.1183/09031936.00112408

Puente-Maestu L, Tejedor A, Lázaro A, *et al*., 2012. Site of mitochondrial reactive oxygen species production in skeletal muscle of chronic obstructive pulmonary disease and its relationship with exercise oxidative stress. *Am J Respir Cell Mol Biol*, 47(3):358-362. https://doi.org/10.1165/rcmb.2011-0382OC

Puente-Maestu L, Lázaro A, Humanes B, 2013. Metabolic derangements in copd muscle dysfunction. *J Appl Physiol (1985)*, 114(9):1282-1290. https://doi.org/10.1152/japplphysiol.00815.2012

Puig-Vilanova E, Rodriguez DA, Lloreta J, *et al*., 2015. Oxidative stress, redox signaling pathways, and autophagy in cachectic muscles of male patients with advanced copd and lung cancer. *Free Radic Biol Med*, 79:91-108. https://doi.org/10.1016/j.freeradbiomed.2014.11.006

Rikka S, Quinsay MN, Thomas RL, *et al*., 2011. Bnip3 impairs mitochondrial bioenergetics and stimulates mitochondrial turnover. *Cell Death Differ*, 18(4):721-731. https://doi.org/10.1038/cdd.2010.146

Roig M, Eng JJ, Macintyre DL, *et al*., 2011. Deficits in muscle strength, mass, quality, and mobility in people with chronic obstructive pulmonary disease. *J Cardiopulm Rehabil Prev*, 31(2):120-124. https://doi.org/10.1097/HCR.0b013e3181f68ae4

Romanello V, Sandri M, 2015. Mitochondrial quality control and muscle mass maintenance. *Front Physiol*, 6:422. https://doi.org/10.3389/fphys.2015.00422

Seymour JM, Ward K, Sidhu PS, *et al*., 2009. Ultrasound measurement of rectus femoris cross-sectional area and the relationship with quadriceps strength in copd. Thorax, 64(5):418-423. https://doi.org/10.1136/thx.2008.103986

Shrikrishna D, Patel M, Tanner RJ, *et al*., 2012. Quadriceps wasting and physical inactivity in patients with copd. Eur Respir J, 40(5):1115-1122. https://doi.org/10.1183/09031936.00170111

Singh AK, Kashyap MP, Tripathi VK, *et al*., 2017. Neuroprotection through rapamycin-induced activation of autophagy and pi3k/akt1/mtor/creb signaling against amyloid-β-induced oxidative stress, synaptic/neurotransmission dysfunction, and neurodegeneration in adult rats. *Mol Neurobiol*, 54(8):5815-5828. https://doi.org/10.1007/s12035-016-0129-3

Spositon T, Oliveira JM, Rodrigues A, *et al*., 2022. Quadriceps weakness associated with mortality in individuals with chronic obstructive pulmonary disease. *Ann Phys Rehabil Med*, 65(5):101587. https://doi.org/10.1016/j.rehab.2021.101587

Su L, Zhang J, Gomez H, *et al*., 2023. Mitochondria ros and mitophagy in acute kidney injury. *Autophagy*, 19(2):401-414. https://doi.org/10.1080/15548627.2022.2084862

Suzuki K, Akioka M, Kondo-Kakuta C, *et al*., 2013. Fine mapping of autophagy-related proteins during autophagosome formation in saccharomyces cerevisiae. *J Cell Sci*, 126(Pt 11):2534-2544. https://doi.org/10.1242/jcs.122960

Swallow EB, Reyes D, Hopkinson NS, *et al*., 2007. Quadriceps strength predicts mortality in patients with moderate to severe chronic obstructive pulmonary disease. *Thorax*, 62(2):115-120. https://doi.org/10.1136/thx.2006.062026

Tashiro H, Takahashi K, Tanaka M, *et al*., 2021. Skeletal muscle is associated with exercise tolerance evaluated by cardiopulmonary exercise testing in japanese patients with chronic obstructive pulmonary disease. *Sci Rep*, 11(1):15862. https://doi.org/10.1038/s41598-021-95413-9

Thériault ME, Paré M, Lemire BB, *et al*., 2014. Regenerative defect in vastus lateralis muscle of patients with chronic obstructive pulmonary disease. *Respir Res*, 15(1):35. https://doi.org/10.1186/1465-9921-15-35

Torres SH, Montes De Oca M, Loeb E, *et al*., 2011. Gender and skeletal muscle characteristics in subjects with chronic obstructive pulmonary disease. *Respir Med*, 105(1):88-94. https://doi.org/10.1016/j.rmed.2010.05.010

Vermeeren MA, Creutzberg EC, Schols AM, *et al*., 2006. Prevalence of nutritional depletion in a large out-patient population of patients with copd. *Respir Med*, 100(8):1349-1355. https://doi.org/10.1016/j.rmed.2005.11.023

Vestbo J, Prescott E, Almdal T, *et al*., 2006. Body mass, fat-free body mass, and prognosis in patients with chronic obstructive pulmonary disease from a random population sample: Findings from the copenhagen city heart study. *Am J Respir Crit*

Unedited

Care Med, 173(1):79-83. https://doi.org/10.1164/rccm.200506-969OC

Wang P, Shao BZ, Deng Z, *et al*., 2018. Autophagy in ischemic stroke. *Prog Neurobiol*, 163-164:98-117. https://doi.org/10.1016/j.pneurobio.2018.01.001

World Health Organization. 2019. Global health estimates: Leading causes of death. Available from https://www.who.int/news-room/fact-sheets/detail/chronic-obstructive-pulmonary-disease-(copd) [Accessed on May. 04, 2023].

Zhang L, Gong H, Sun Q, *et al*., 2018. Spermidine-activated satellite cells are associated with hypoacetylation in acvr2b and smad3 binding to myogenic genes in mice. *J Agric Food Chem*, 66(2):540-550. https://doi.org/10.1021/acs.jafc.7b04482

Zorov DB, Juhaszova M, Sollott SJ, 2014. Mitochondrial reactive oxygen species (ros) and ros-induced ros release. *Physiol Rev*, 94(3):909-950. https://doi.org/10.1152/physrev.00026.2013

unedited