HIGHLIGHTED TOPIC | Muscle Dysfunction in COPD

Role of autophagy in COPD skeletal muscle dysfunction

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Hussain SN, Sandri M. Role of autophagy in COPD skeletal muscle dysfunction. J Appl Physiol 114: 1273–1281, 2013. First published October 18, 2012; doi:10.1152/japplphysiol.00893.2012.—Chronic obstructive pulmonary disease (COPD) is a debilitating disease caused by parenchymal damage and irreversible airflow limitation. In addition to lung dysfunction, patients with COPD develop weight loss, malnutrition, poor exercise performance, and skeletal muscle atrophy. The latter has been attributed to an imbalance between muscle protein synthesis and protein degradation. Several reports have confirmed that enhanced protein degradation and atrophy of limb muscles of COPD patient is mediated in part through activation of the ubiquitin-proteasome pathway and that this activation is triggered by enhanced production of reactive oxygen species. Until recently, the importance of the autophagy-lysosome pathway in protein degradation of skeletal muscles has been largely ignored, however, recent evidence suggests that this pathway is actively involved in recycling of cytosolic proteins, organelles, and protein aggregates in normal skeletal muscles. The protective role of autophagy in the regulation of muscle mass has recently been uncovered in mice with muscle-specific suppression of autophagy. These mice develop severe muscle weakness, atrophy, and decreased muscle contractility. No information is yet available about the involvement of the autophagy in the regulation of skeletal muscle mass in COPD patients. Pilot experiments on vastus lateralis muscle samples suggest that the autophagy-lysosome system is induced in COPD patients compared with control subjects. In this review, we summarize recent progress related to molecular structure, regulation, and roles of the autophagy-lysosome pathway in normal and diseased skeletal muscles. We also speculate about regulation and functional importance of this system in skeletal muscle dysfunction in COPD patients.

skeletal muscles; chronic obstructive pulmonary disease; mammalian target of rapamycin; forkhead box O transcription factors; atrophy; muscle wasting

MUSCLE ATROPHY IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE PATIENTS

It has long been recognized that chronic obstructive pulmonary disease (COPD) is associated with skeletal muscle dysfunction. Functionally, skeletal muscle dysfunction in COPD is characterized by significant reductions in muscle strength and endurance (24). Structurally, it is characterized by decreases in muscle mass and cross-sectional area (muscle atrophy), alterations in fiber-type distribution (decreased proportions of oxidative fibers and increased proportions of glycolytic fibers), changes in oxidative metabolic capacity (attenuated mitochondrial enzyme activities and expressions), and modifications in capillary distribution (significantly decreased capillary density).

The reductions in mass and cross-sectional area of limb muscles of COPD patients have been linked to reduced muscle strength seen in these patients. When limb muscle strength is normalized per unit of mass or cross-sectional area, no differences can be observed between control subjects and COPD patients (4), thereby suggesting that atrophy is indeed an important causative factor in reduced limb muscle strength and endurance in COPD. However, in addition to atrophy, intrinsic abnormalities inside limb muscle fibers of COPD patients may also contribute to the contractile fatigue that is seen in these muscles, particularly in response to physical exercise (41, 58).

PROTEOLYSIS IN SKELETAL MUSCLES

Regulation of muscle mass is a dynamic process that involves a delicate balance between hypertrophic (protein synthesis) and atrophic (degradation) signaling pathways. In COPD patients, it is generally assumed that the balance is tilted toward reduced protein synthesis and enhanced protein degradation. The precise mechanisms underlying this imbalance...
remain unclear although there has been speculation that activation of catabolic factors such as interleukin-6 (IL-6) and cortisol and inhibition of anabolic factors such as testosterone, dehydroepiandrosterone, and insulin-like growth factor 1 (IGF-I) are involved (15). Other explanations implicate local factors, such as oxidative stress caused by increased reactive oxygen species (ROS), or systemic factors, such as inflammation, malnutrition, corticosteroid therapy, inactivity, smoking, aging, and hypoxemia (13).

Protein degradation in skeletal muscles takes place via four proteolytic systems, including the ubiquitin-proteasome (UP) pathway, the calpain pathway, the caspase pathway, and the autophagy-lysosome (AL) pathway. In various models of muscle wasting, there is coordinated stimulation of these pathways. The most prevalent view of skeletal muscle proteolysis holds that the initial step in myofilament protein degradation is disruption of the myofibrillar assembly by Ca^{2+}-dependent calpains and caspases (cystein proteases), so as to enable the UP pathway in its main task of degrading contractile proteins (70). The primary role of the AL pathway is to recycle cytoplasmic proteins and organelles, including the mitochondria and peroxisomes.

**UP PATHWAY**

The UP pathway has long been considered to be the primary system responsible for degradation of the bulk of myofilament proteins and some membrane proteins (70). This ATP-dependent proteolytic system is extremely complex and involves a cascade of enzymatic reactions in which proteins that are targeted for degradation go through several steps, the first of which involves the activation of ubiquitin by E1 ubiquitin-activating proteins. Activated ubiquitin is then transferred to E2 ubiquitin-conjugating enzymes and, finally, transferred by E3 ligases to the target proteins. Following the ubiquitination process, selected proteins are unfolded and delivered to the 26S proteasome, which consists of a 20S catalytic core and two 19S regulatory caps. Chymotrypsin-like, trypsin-like, and caspase-like activities of the 20S core are tasked with cleaving the tagged proteins into short oligopeptides that then undergo further degradation by cytoplasmic peptidases while the ubiquitin is being recycled.

In various models of atrophy, including denervation, immobilization, and fasting, recent studies have identified two E3 ligases that are expressed specifically and abundantly in skeletal muscles: muscle ring finger 1 (MURF1) and muscle atrophy F-box (MAFbx or ATROGIN-1) (5, 23). The expressions of MURF1 and ATROGIN-1 are induced by a complex signaling network consisting of forkhead box O (FOXO) 1, FOXO3, and nuclear factor-κB (NF-κB) transcription factors (6, 60). It has also been demonstrated that activation of the IGF-1phosphatidylinositol 3-kinase/protein kinase B (AKT) pathway, which is essential for muscle cell survival and hypertrophy, leads to inhibition of MURF1 and ATROGIN-1 (65). Once activated, AKT phosphorylates FOXO1 and FOXO3 proteins. They are then excluded from the nucleus and become transcriptionally inactive (65). Upstream signals that activate the UP pathway under conditions of atrophy are as yet still under investigation.

In skeletal muscles, multiple stimuli are likely to activate the proteasome and lead to enhanced degradation of myofilament proteins. One such stimulus is an increased level of ROS. In cultured skeletal muscle cells, exposure to ROS triggers significant increases in proteasomal activity and enhanced expressions of MURF1 and ATROGIN-1 (40). Similarly, in cultured cells and in vivo intact muscles, the proinflammatory cytokine tumor necrosis factor-α (TNF-α) and NF-κB transcription factor have been shown to augment the activity of the 20S proteasome and to trigger significant inductions of MURF1 and ATROGIN-1 (6, 39). Furthermore, in cultured skeletal myotubes, hypoxia has been shown to be a strong activator of 20S activity and ATROGIN-1 expression (7). Evidence that supports increased UP activity in limb and ventilatory muscles of COPD patients is largely based on measuring 20S proteasomal activity and documenting the expression levels of MURF1 and ATROGIN-1 (19, 54). NEDD4 (a HECT domain E3 ligase) has also been shown to be significantly elevated in these patients (55). Elevated E3 ligase expression is likely to be the result of increased transcriptional activity of FOXO transcription factors (14, 19).

**CALPAINS AND CASPASES**

Calpains consist of 14 different cysteine proteases that are dependent on Ca^{2+}. Skeletal muscles contain substantial levels of three distinct calpains: the ubiquitous calpains 1 and 2 (μ- and m-calpains), which require micromolar and millimolar concentrations of Ca^{2+} for their activity, and calpain 3, which is mainly expressed in skeletal muscles (2). Calpains are highly regulated and are inactive most of the time. Their activities are regulated by Ca^{2+} spikes, phosphorylation, and association with the selective inhibitor calpastatin (2). Calpains cleave proteins at selective sites and are not involved in the degradation of cytosolic proteins inside skeletal muscles. Caspases are responsible for degrading cellular proteins during apoptosis. Many recent reports have documented increases in caspase activity and/or expression inside skeletal muscles in a variety of pathological conditions, including muscular dystrophy, sepsis, and exercise-induced oxidative stress (37, 66). Although it is known that caspase-3 acts upstream from the UP pathway and that it is capable of degrading actin in vitro settings, the exact contributions of caspases to in vivo skeletal muscle protein degradation remain unclear.

**AL PATHWAY**

The AL pathway consists of a delivery system (autophagy) and a catalytic component (lysosomes). Autophagy is an evolutionarily conserved pathway of self-digestion that occurs in eukaryotic organisms. It uses the lysosomal machinery for degradation and recycling of bulk cytoplasm, long-lived proteins, and organelles. Therefore, autophagy can be defined as a process of cytosolic “renovation” (46). Three different delivery systems have been described in mammals: microautophagy, chaperone-mediated autophagy (CMA), and macroautophagy. The term microautophagy applies to processes in mammalian cells that involve the presence of lysosome-like organelles that have multiple vesicles trapped in their lumen. Currently, microautophagy is considered the form of autophagy that is responsible for the degradation of long-lived proteins. This is achieved through sequestration of cytosolic cellular constituents by protrusions of lysosomal membrane. Little is known about underlying mechanisms, regulation, or physiological...
significance of microautophagy in mammalian cells. Studies in the late 1970s and 1980s on hepatocytes and cardiac muscle cells revealed some evidence of the existence of microautophagy in mammalian cells, including the presence of microautophagic activity in vitro isolated lysosomes, visualization under electron microscopy of a lysosomal wrapping mechanism, and the presence of starvation-induced microautophagic vacuoles (for review, see Ref. 45). Recently, Takikita et al. (67) have postulated microautophagy as a possible mechanism by which excess glycogen is taken up by lysosomes of skeletal muscle cells.

In CMA, substrates are comprised of soluble proteins that are recognized by a complex that is constituted mainly by heat shock protein 70 (HSP70) and LAMP2A protein (3). Only those proteins that contain a specific sequence of five amino acids (KFERQ), estimated to be present in only 25–30% of all cell proteins, are recognized. When a protein is damaged, the KFERQ motif is exposed and recognized by the HSP70-LAMP2 complex. It then undergoes complete unfolding and degradation by the lysosomes. Under normal conditions, CMA occurs relatively infrequently. However, upon starvation or amino acid deprivation, CMA is activated within a few hours and even substitutes for macroautophagy as a source of amino acids (3). Starvation-induced CMA has been documented in the liver, heart, kidneys, and spleen (12). No information is as yet available regarding the involvement of CMA in protein recycling of skeletal muscle cells. However, in LAMP2-deficient mice, extensive accumulation of autophagic vacuoles occurs in many tissues, including the skeletal muscles (68), cardiac myocytes are structurally abnormal, and heart contractility is severely compromised, confirming that LAMP2 protein is critical to autophagy in many organs, including the heart and skeletal muscles (68).

Macroautophagy (synonymous with autophagy in this review) is characterized by the formation of double-membrane vesicles known as autophagosomes that surround portions of cytoplasm, organelles, glycogen, and protein aggregates. Autophagosomes are then delivered to lysosomes for degradation of their contents to provide amino acids. This process consists of a cascade of tightly regulated steps, from membrane commitment and elongation to autophagosome formation and fusion with the lysosomes (Fig. 1). These steps rely upon a number of so-called autophagy-related proteins (ATG), first identified in yeast, which involve two ubiquitin-like conjugation systems corresponding to the ATG12-ATG5 and ATG8-phosphatidylethanolamine (PE) complexes, as well as additional ATG regulatory complexes (53). Most ATG orthologs have been isolated in mammals, and ATG inactivation in the mouse has revealed interesting phenotypes (38).

![Fig. 1. Autophagosome formation and autophagy-related proteins (Atg) in mammalian cells. PI3P, phosphatidylinositol-3-phosphate; DCP1, double FYVE-containing protein1; WIPI, WD-repeat protein interacting with phosphoinoside (Atg18 homolog); PE, phosphatidylethanolamine; AMPK, AMP-activated protein kinase; ULK1, Unc-51-like kinase 1; mTORC1, mammalian target of rapamycin complex 1; FIP200, FAK family-interacting protein of 200 kDa; Bcl2, B-cell lymphoma 2; Beclin1, Bcl2 interacting protein 1; Ambra1, activating molecule in Beclin1-regulated autophagy 1; Vps34, PI3P kinase (PI3K) class III. [Modified from Mizushima and Komatsu (46).]]
Autophagy is initiated by the ULK1-ATG13-FIP200 complex (Fig. 1). This complex is under the control of the AMP-activated protein kinase (AMPK) and complex 1 of mammalian target of rapamycin (mTORC1) pathways (Fig. 1). In nutrient-rich conditions, mTORC1 phosphorylates and inactivates ULK1, thereby inhibiting autophagy (34). In response to nutrient deprivation, AMPK senses low nutrient and energy status, inactivates the mTORC1 complex, and directly phosphorylates ULK1 on several serine residues (21, 34). This initiates autophagosome formation (Fig. 1). Initiation of autophagy is followed by a membrane nucleation process that is dependent on the BECLIN1 complex (BECLIN1, VPS34, VPS15, UVRAG, and ATG14L) (Fig. 1). Membrane formation proceeds by sequestration of small ubiquitin-like molecules, including LC3B, GABARAP, GATE-16, and ATG12. LC3B, GABARAP, and GATE-16 are conjugated by covalent binding to the phospholipid PE, whereas ATG12 binds to ATG5 (69). The lives of these molecules and their homologs are very short (<40 min) (30, 64). A bound molecule is transferred to the membrane; the membrane grows and then becomes a double-membraned vesicle, or autophagosome (35). Covalent binding occurs on both the inner and outer membranes of the autophagosome. Phospholipid sources for the growing autophagosome include the endoplasmic reticulum, mitochondria, and plasma membranes. Organelles and proteins that have been sequestered by the autophagosome are then docked to a lysosome for degradation. Fusion of the autophagosome to the lysosome leads to degradation of the inner membrane of the autophagosome, including any proteins that are attached to it.

According to guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes (36), multiple assays are required to quantify the degree of autophagy in a given cell or organ. These include direct measurement of autophagic protein degradation using radioactive amino acids, monitoring autophagosome formation with electron microscopy, assessing the conversion of LC3B-I to LC3B-II, detecting LC3B incorporation into the autophagosomes using fluorescence microscopy, monitoring ULK1 (ATG1) kinase activity, assessing autophagy-related gene expression, and monitoring autophagy by flux measurements, where LC3B-II turnover is measured following inhibition of lysosomal degradation (36).

Early studies on autophagy suggested that the process is primarily a nonselective degradation pathway. However, it has become increasingly evident in recent years that several selective forms of autophagy exist. One particular organelle that is selectively targeted by autophagy is the mitochondrion. The process of recycling mitochondria by autophagy is known as mitophagy, although the mechanisms by which mitochondria are selectively recycled are not clearly understood. Recent studies have indicated that reduction of mitochondrial membrane potential (ΔΨm) by an exogenous uncoupling agent leads to autophagic mitochondrial removal (50). Physiologically, reduction of ΔΨm can be induced by opening of the permeability transition pore (PTP), a channel that regulates ΔΨm and mitochondrial Ca2+ buffering capacity and that is formed, in part, by cyclophilin D. In mammals, several proteins, including PINK1, PARKIN, BNIP3, and BNIP3L, have recently been shown to play critical roles in the relationship between reduction of ΔΨm and mitophagy.

PINK1 is a mitochondrial kinase that under normal conditions is rapidly degraded by mitochondrial proteases (74). PINK1 degradation is inhibited in depolarized mitochondria, stabilized on the mitochondrial outer membrane, and followed by recruitment of the E3 ligase PARKIN. Accumulation of PARKIN causes ubiquitination of outer mitochondrial membrane proteins recognized by SQSTM1 (p62), an adaptor protein that transports autophagic vesicles to ubiquitinated mitochondrial proteins. BNIP3 (Bcl2 adenovirus E1B 19 kDa-interacting protein 3) and its homolog BNIP3L (NIX) are proapoptotic proteins that have recently been identified as important regulators of both apoptosis and mitophagy. They have several features in common with the BH3-only proteins of the Bcl2 family. They contain BH3 domains, localize to the mitochondrial membrane, activate downstream effectors such as BAX and BAK, and interact with the anti-apoptotic Bcl2 and BclXL.

Recent studies have demonstrated that both BNIP3 and BNIP3L induce removal of mitochondria via autophagy and that this process is independent of changes in PTP opening; it is accomplished through direct binding of LC3B, which is followed by recruitment of growing autophagosomes to the mitochondrion (28, 51). BNIP3L appears to be essential for autophagic mitochondrial clearance during reticulocyte maturation (63), whereas BNIP3 is involved in mitochondrial recycling in cardiac myocytes (27). In atrophying muscle, the mitochondrial network is dramatically remodeled following fasting or denervation and BNIP3-mediated autophagy contributes to this remodeling (57). Expression of the fission machinery per se was sufficient to cause muscle wasting in mice by causing organelle dysfunction and the activation of AMPK, whereas inhibition of fission prevents muscle loss during denervation, indicating that disruption of the mitochondrial network is a critical amplificatory loop of the muscle atrophy program (57). Conversely, impairment of basal mitophagy is deleterious to muscle homeostasis and leads to the accumulation of damaged and dysfunctional mitochondria (25).

**AUTOPHagy IN SKELETal MUSCles**

Despite the fact that autophagy has been described in all cell types, its role in skeletal muscle protein degradation has been largely ignored. Recent work by Masiero et al., however, has demonstrated that basal autophagy is critical to muscle homeostasis, since it is responsible for the removal of protein aggregates and damaged mitochondria (43). It has also been shown that autophagy is significantly induced in limb muscles in response to denervation (32, 52).

In kinetic terms, stress-induced autophagy in skeletal muscle differs compared with other tissues. In response to 24 and 48 h of starvation, most nonmuscle tissues undergo induction of autophagy, which peaks after 24 h of starvation, and little autophagy can be detected after 48 h of starvation. In contrast, persistent generation of autophagosomes continues for 48 h of starvation in skeletal muscles (47) (Fig. 2). This prolonged induction is made possible by a unique FOXO transcriptional program that works to replenish the short-lived proteins that are required for autophagosome-lysosome fusion (42). In functional terms, autophagic regulation of skeletal muscle mass has recently been elucidated by selective deletion of ATG7, a unique E1 enzyme of the autophagic machinery. Muscle-
specific deletion of this enzyme leads to complete inhibition of autophagosome formation, accumulation of abnormal mitochondria and polyubiquitinated proteins, oxidative stress, disorganized sarcomeres, and activation of the unfolded protein response, which, in turn, trigger myofiber degeneration (43). Moreover, the muscles of ATG7-deficient mice develop severe weakness and atrophy and display several other signs of myopathy. Suppression of autophagy exacerbates fasting- and denervation-induced atrophy in these mice (44).

A similar phenotype to that of muscle-specific ATG7-deficient mice has been observed in mice with muscle-specific ablation of ATG5, another critical component of the autophagic machinery (56). Recent work has also revealed that nutrient-deprivation autophagy factor-1 (Naf-1), a Bcl2-associated autophagy regulator, is required for the homeostatic maintenance of skeletal muscle. Naf-1-deficient mice exhibit muscle weakness and markedly decreased strength, accompanied by increased autophagy, dysregulation of calcium homeostasis, and enlarged mitochondria (8).

Histone deacetylases (HDACs) are also critical regulators of starvation-induced autophagy in skeletal muscles. Muscle-specific deletion of both HDAC1 and HDAC2 results in partial perinatal lethality, and HDAC1/2 knockout mice that survive postnatally develop progressive myopathies characterized by abnormal muscle degeneration/regeneration and metabolism that were associated with autophagy blockade (48).

An important link between physical exercise and autophagy in skeletal muscles has very recently come to light. Grumati et al. (26) report that physical exercise is very effective in stimulating autophagy in skeletal muscles and that the accompanying clearance of damaged cell components and dysfunctional mitochondria is beneficial for muscle homeostasis. Moreover, He et al. (31) have evaluated the importance of exercise-induced autophagy in regulating metabolism by generating mice (Bcl2 AAA) that contain knock-in mutations in Bcl2 phosphorylation sites that prevent exercise-induced disruption of the Bcl2-BECLIN1 complex and autophagy activation. Bcl2 AAA mice develop with significantly decreased exercise endurance, altered glucose metabolism during exercise, and impaired exercise-induced protection against high-fat diet-induced glucose intolerance (31). These findings underline the importance of autophagic regulation of the beneficial metabolic effects of exercise.

**AUTOPHAGY IN COPD PATIENTS**

The functional importance of autophagy in the pathogenesis of lung disease in COPD patients has recently been demonstrated by Chen et al. (10) who described significant increases of autophagy in clinical lung samples taken from COPD patients. LC3B, BECLIN1, ATG7, and ATG5 were all upregulated, and autophagosome formation was visualized using electron microscopy. Similar evidence of increased autophagy was observed in mice subjected to chronic inhalation of cigarette smoke (10) and in lung epithelial cells exposed to aqueous cigarette smoke extracts (33). These studies suggest that autophagy is activated as a stress response. However, more complete elucidation of the functional roles of autophagy in the etiology of pulmonary dysfunction in COPD patients remains to be determined.

To date, little research has been completed on the contribution of the AL system, per se, to protein degradation and loss of skeletal muscle mass in COPD patients. To our knowledge, only Plant et al. (55) have addressed these issues. They examined vastus lateralis muscle biopsies obtained from nine control subjects and nine patients with severe COPD [forced expiratory volume in 1 s (FEV1) values of 35 ± 2% of predicted] and muscle atrophy (quadriceps cross-sectional areas of 61 ± 5
cm²). ATROGIN-1 and NEDD4 expressions were significantly elevated while that of MURF1 was similar to what was observed in control subjects. They assessed autophagy by measuring mRNA levels of BECLIN1 and LC3B and found that they were not significantly different compared with controls. Based on these results, Plant et al. concluded that autophagy is not activated in muscles of COPD patients. However, it should be emphasized that relatively few patients were studied by these authors and that they only measured mRNA levels of BECLIN1 and LC3B to determine the degree of autophagy. It is entirely conceivable that autophagy can be mildly activated with no accompanying upregulation of BECLIN1 and LC3B.

To assess whether or not autophagy is indeed activated in limb muscles of COPD patients, we performed pilot experiments using immunoblotting and real-time PCR mRNA measurements to evaluate autophagy-related gene expression in vastus lateralis muscle biopsies obtained from patients with severe COPD (FEV₁ values ranging between 51 and 19% of predicted) and control subjects matched for age and physical activity. These experiments revealed significant increases in the intensity of LC3B-II protein (marker of autophagosomes) in muscle biopsies of COPD patients compared with control subjects. This observation could be the result of a significant increase in autophagosome formation or impaired autophagosome degradation by the lysosomes. Both mechanisms can lead to a rise in LC3B-II protein intensity. However, the fact that we also observed significant increases in BECLIN1 and SQSTM1 (p62) protein levels in muscle biopsies of COPD patients compared with control subjects suggests that autophagy is significantly induced in vastus lateralis muscle of COPD patients. On the basis of these preliminary experiments, we speculate that enhanced basal autophagy may contribute to skeletal muscle remodeling in skeletal muscles of COPD patients.

**FACTORS THAT MAY REGULATE AUTOPHAGY IN MUSCLES OF COPD PATIENTS**

We propose that, if autophagy is activated in skeletal muscles of COPD patients, local and systemic factors such as oxidative stress, inflammation, malnutrition, therapeutic medication, and aging may be involved (Fig. 3).

Several reports have confirmed that autophagy is activated by oxidative stress (9, 61). A recent study, in which ROS levels in skeletal muscles were selectively increased, demonstrates that substantial induction of autophagy causes severe muscle atrophy (18). ROS influence autophagy through several mechanisms of action, including: 1) activation of AMPK and inhibition of mTORC1 (61); 2) recruitment of BNIP3 to the mitochondria as a Bcl2-binding competitor of BECLIN1, a trigger of autophagy (61); 3) activation of FOXO transcription factors, which regulate many autophagy-related genes, including LC3B, GABARAP, and BNIP3 (76); and 4) activation of the mitochondrial PTP, which results in loss of membrane potential and recruitment of PARKIN and autophagosomes to the mitochondria (49).

Inflammatory mediators are also likely to play a role in the induction of autophagy in skeletal muscles of COPD patients (Fig. 3). Systemic and/or local pulmonary inflammation has been identified as a common feature of COPD. In stable COPD patients, elevated serum levels of C-reactive protein, fibrinogen, circulating leukocytes, and proinflammatory cytokines, including TNF-α, interleukin-8, IL-6, and soluble TNF receptors p55 and p75 have been observed (17, 22, 62). Cytokines such as TNF-α, interleukin-1, IL-6, and ligands of Toll-like receptor 4 have recently been shown to induce autophagy in cardiac and skeletal muscles (20, 29, 75).

Induction of basal autophagy in skeletal muscles of COPD patients might also be a response to malnutrition that develops as a result of an imbalance between energy intake and energy expenditures (Fig. 3). Reduced dietary intake has been attributed to symptoms such as postprandial dyspnea, early satiety, fatigue, and loss of appetite. Elevated energy expenditures have been attributed to increased work of breathing, thermogenic effects of bronchodilators, and systemic inflammation (1, 71). Malnutrition is likely to stimulate autophagy in skeletal muscles through simultaneous activation of the AMPK pathway and inhibition of the mTORC1 pathway, with both responses causing activation of ULK1 catalytic activity (ATG1) and initiation of autophagy (59).

Short periods of high-dose corticosteroid therapy and prolonged periods of low-dose corticosteroid therapy are commonly prescribed for acute exacerbations of COPD and for controlling chronic symptoms in some patients. Long-term...
therapy with relatively high doses of corticosteroids elicits significant reductions in strength and atrophy of both ventilatory and limb muscles of COPD patients (16). The effects of corticosteroid therapies on the regulation of autophagy in skeletal muscles remain unclear, although it is known that corticosteroids such as prednisolone trigger significant inductions of autophagy in other cell types (73). It is conceivable that prednisolone therapy might evince a similar effect in the skeletal muscles of COPD patients.

Senescence, or aging, is another important factor that should be taken into consideration when assessing the contribution of autophagy to the regulation of skeletal muscle proteolysis in COPD patients. It has been proposed that a chronic decline in autophagy may be a major factor in the excessive loss of muscle mass that occurs in the elderly. Depressed autophagy in aged individuals may interfere with the contractile properties of myofibers and render them less stable and more susceptible to contraction-induced damage, eventually leading to muscle atrophy (11). Few studies have investigated the process of autophagy in the skeletal muscles of aged humans, however, recent data confirm that imbalanced autophagy may contribute to loss of skeletal muscle mass in the elderly. Wohlgemuth et al. (72) described a significant decline in limb muscle autophagy despite an increase in BECLIN1 expression. Attenuation of autophagy was associated with oxidative damage and apoptosis, confirming the protective role that autophagy plays in skeletal muscles, as previously described by our group (43). This protective role was also confirmed by showing that caloric restriction ameliorates oxidative damage in skeletal muscles of aged rats (72). These findings suggest that the protective role of autophagy in elderly COPD patients may be attenuated compared with young patients and that this attenuation may contribute to the development of skeletal muscle atrophy and enhanced apoptosis in aged COPD patients.

FUTURE PERSPECTIVES

It is clear that accurate quantification of the extent of autophagy in limb and ventilatory muscles of COPD patients is very much needed at this time. The completion of multiple assays aimed at reliably monitoring the many complex stages involved in autophagosome formation and degradation will be required. Assays such as those that measure LC3B-II protein intensities and mRNA and protein levels of critically important autophagy-related genes are readily available, as are electron microscopy techniques that quantify the number and volume of autophagic elements, such as autophagosomes and lysosomes. Direct measurements of long-lived protein degradation will need to be further developed for relatively small human skeletal muscle biopsies (36). Furthermore, until recently, it has been very difficult to directly measure autophagic flux in vivo. New assays, however, such as those that utilize leupeptin or colchicine injection, have been extremely beneficial to animal research although they have not yet been validated for use in humans.

In the future, creative use of a wide array of techniques should enable researchers to investigate the complex relationships that exist between autophagy, morphology, and skeletal muscle dysfunction in patients with COPD. Topics such as muscle fiber switching, atrophy, reduced strength, and compromised endurance all need to be addressed in relation to autophagy. More specifically, it is important to address the question of whether autophagy in COPD patients contributes to muscle atrophy and contractile impairment at the functional level. This is a timely question because optimal regulation of autophagic flux is a critical factor in the maintenance of skeletal muscle homeostasis, both in normal conditions and in response to stress. If it is defective, excessive, or otherwise abnormal, autophagy plays a pathogenic role and is extremely harmful to muscle health.

It is possible that autophagic flux is excessively induced in COPD as a result of local and systemic factors and that this contributes to skeletal muscle atrophy. It may also be that disproportionate removal of autophagosome-targeted cellular components by autophagy occurs. Mitochondria are targeted in this way and it is indeed plausible that the significant reductions in skeletal muscle mitochondrial contents that are observed in patients with severe COPD may be due to excessive mitophagy. Conversely, it may also be possible that autophagy is insufficiently induced in skeletal muscles of COPD patients, thereby precipitating significant impairments in myofiber homeostasis as a consequence of damaged or dysfunctional cell component accumulation.

At present, however, it is difficult to conclusively determine whether autophagy is excessively induced or whether it is inadequately upregulated in COPD. This is due to a marked paucity in the kind of extensive morphological, biochemical, and molecular documentation that is required to evaluate the relationship between extent of autophagy and COPD. Furthermore, experiments that assess muscle structure and function in response to selective inhibition of autophagy have not as yet been performed on COPD muscle samples, chiefly due to the fact that safe and effective selective autophagy inhibitors, particularly those that do not directly influence pathways that are involved in protein recycling or synthesis, are unavailable.

In time, however, knowledge of the molecular mechanisms of autophagy will be more deeply and widely understood. Better and more accurate techniques will be devised. Such developments will be crucial to the overall project of understanding how autophagy relates to COPD and to the successful development of therapies to counter this difficult disease.

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