Skeletal muscle mitophagy in chronic disease: implications for muscle oxidative capacity?

Skeletal muscle mitophagy in chronic disease: implications for muscle oxidative capacity?

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Purpose of review
Loss of skeletal muscle oxidative capacity is a common feature of chronic diseases such as chronic obstructive pulmonary disease, type 2 diabetes, and congestive heart failure. It may lead to physical impairments and has been suggested to contribute to metabolic inflexibility-induced cardiometabolic risk. The mechanism underlying loss of muscle oxidative capacity is incompletely understood. This review discusses the role of mitophagy as a driving force behind the loss of skeletal muscle oxidative capacity in these patients.

Recent findings
Mitophagy has been studied to a very limited extent in human skeletal muscle. There are, however, clear indications that disease-related factors, including hypoxia, systemic inflammation, muscle inactivity, and iron deficiency are able to induce mitophagy, and that these factors trigger mitophagy via different regulatory mechanisms. Although mitophagy may lead to mitochondrial loss, it is also required to maintain homeostasis through clearance of damaged mitochondria.

Summary
Based on available evidence, we propose that enhanced mitophagy is involved in chronic disease-induced loss of muscle oxidative capacity. Clearly more research is required to confirm this role and to establish to what extent mitophagy is pathological or a part of physiological adaptation to maintain muscle health.

Keywords
chronic obstructive pulmonary disease, congestive heart failure, mitophagy, skeletal muscle, type 2 diabetes

INTRODUCTION

Loss of lower limb skeletal muscle oxidative capacity, defined by the ability to oxidize nutrients to obtain energy, is a common feature of chronic diseases such as chronic obstructive pulmonary disease (COPD) [1], type 2 diabetes (T2D) [2,3], and congestive heart failure (CHF) [4,5]. Functional impairments, such as muscle dysfunction and decreased exercise capacity, are associated with reduced muscle oxidative capacity [1–5], and eventually may lead to disability or even handicaps. Moreover, impaired muscle oxidative capacity has been proposed to accelerate muscle wasting [6▪▪,7] and may lead to metabolic inflexibility and increased cardiometabolic risk [8].

Oxidative capacity is defined by the maximal rate at which oxidative phosphorylation, a mitochondrial-based process in which energy is obtained by splitting nutritional substrates into CO_2 and water, can be performed. In short, an energy-rich proton gradient is created over the inner mitochondrial membrane (IMM) by pumping protons into the intermembrane space using energy derived from splitting substrates. This proton gradient then drives the production of ATP from ADP and inorganic phosphate. Oxidative phosphorylation was traditionally considered to be the only major function of mitochondria, but recent studies have revealed highly regulated roles for mitochondrial quality and quantity in key cellular regulatory pathways like apoptosis and autophagy [6▪▪].

As skeletal muscle mitochondrial quantity and as such oxidative capacity are clearly affected in

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KEY POINTS

- Skeletal muscle oxidative capacity loss is a common feature of chronic diseases such as COPD, T2D, and CHF.
- Mitochondrial homeostasis is regulated by the balance between mitochondrial biogenesis and mitochondrial breakdown.
- Disease-related factors such as hypoxia, inflammation, muscle disuse, and iron deficiency each in itself have mitophagy-inducing potential, although data in skeletal muscle are lacking.
- Mitophagy targets both healthy and dysfunctional mitochondria and future research should reveal whether skeletal muscle mitophagy is a pathological feature of chronic disease or merely a physiological adaptation.

chronic diseases, it is likely that mitochondrial homeostasis is altered in such a way that mitochondrial degradation, which is mainly mediated through mitophagy, is favored above mitochondrial biogenesis. The main focus has been on impaired skeletal muscle mitochondrial biogenesis in patients with chronic disease in the past decades [9–13], but remarkably, the role of mitochondrial breakdown remains understudied. This review aims to examine current evidence of skeletal muscle mitophagy in different chronic diseases, and to examine evidence linking common denominators of these diseases to increased mitophagy and the loss of skeletal muscle oxidative capacity. Finally, the putative role of skeletal muscle mitophagy in maintenance of muscle health will be discussed.

MITOCHONDRIAL DYNAMICS

According to popular belief, the mitochondrion used to be a prokaryote, which was engulfed by endocytosis in early eukaryotes millions of years ago. Today, mitochondria still contain their own DNA (mtDNA), which consists of 37 genes which are coding for several different mitochondrial proteins, protein subunits, or supporting molecules required for mitochondrial function. The remaining mitochondrial proteins and subunits are encoded by nuclear DNA. To ensure coordinated transcription of both the mtDNA and nuclear DNA, the peroxisome proliferator-activated receptor-γ coactivator family acts as mitochondrial biogenesis master regulator [14].

Mitochondria are highly dynamic organelles, which are constantly changing in size and shape. These changes are mediated through mitochondrial fission and fusion events [6▪▪]. Dynamin-related protein 1 is master regulator for mitochondrial fission, and works together with proteins like mitochondrial fission 1 and mitochondrial fission process 18. Mitochondrial fusion is mainly regulated on the level of the IMM by optic atrophy 1, and on the outer mitochondrial membrane (OMM) by mitofusin (MFN)-1, and MFN-2 [6▪▪]. Due to the selective regulation of these processes, mitochondria in one cell can be highly heterogeneous in size, function, and morphology, and mtDNA copy number.

One of the functions of fusion is to 'dilute' damaged mtDNA or proteins in large mitochondrial networks to enable mitochondrial damage repair. However, because of their exposure to high concentrations of reactive oxygen species (ROS), mitochondria are highly susceptible for protein and mtDNA damage, which can easily become very extensive [15]. To ensure overall mitochondrial health, the mitochondrial network is able to direct damaged mtDNA and proteins into a specific mitochondrial area, which can then be separated from the main mitochondrial network via mitochondrial fission and broken down via mitophagy [6▪▪].

There are three different levels for clearance of mitochondrial content. The first is the mitochondrial ubiquitin-proteasome system, where specific (damaged) proteins are targeted for destruction without influencing the function of the mitochondria [6▪▪]. Second, there is the formation and off-budding of small mitochondrial-derived vesicles, which are subsequently cleared via lysosomal breakdown [6▪▪]. Finally, mitochondrial autophagy (mitophagy) clears complete or large pieces of mitochondria, and is therefore an important regulator of mitochondrial quantity.

MITOPHAGY

Mitophagy initiation is generally divided in, but is not exclusive to, two main pathways which are extensively reviewed in other studies [14,16▪,17,18]. Although these pathways are often described separately, crosstalk between these pathways has been shown. Figure 1 depicts a short summary of the different mitophagy pathways, and their most important players, as described below.

The first pathway is the receptor-based mitophagy pathway. This pathway selectively targets specific mitochondria by post-translational activation of OMM bound mitophagy receptor proteins. By activation of these receptor proteins [i.e., BCL2/adenovirus E1B 19 kDa protein-interacting protein (BNIP)3, BNIP3L, FUN14 domain-containing protein 1 (FUNDC1)], their binding to an autophagosomal-specific protein of the microtubule-

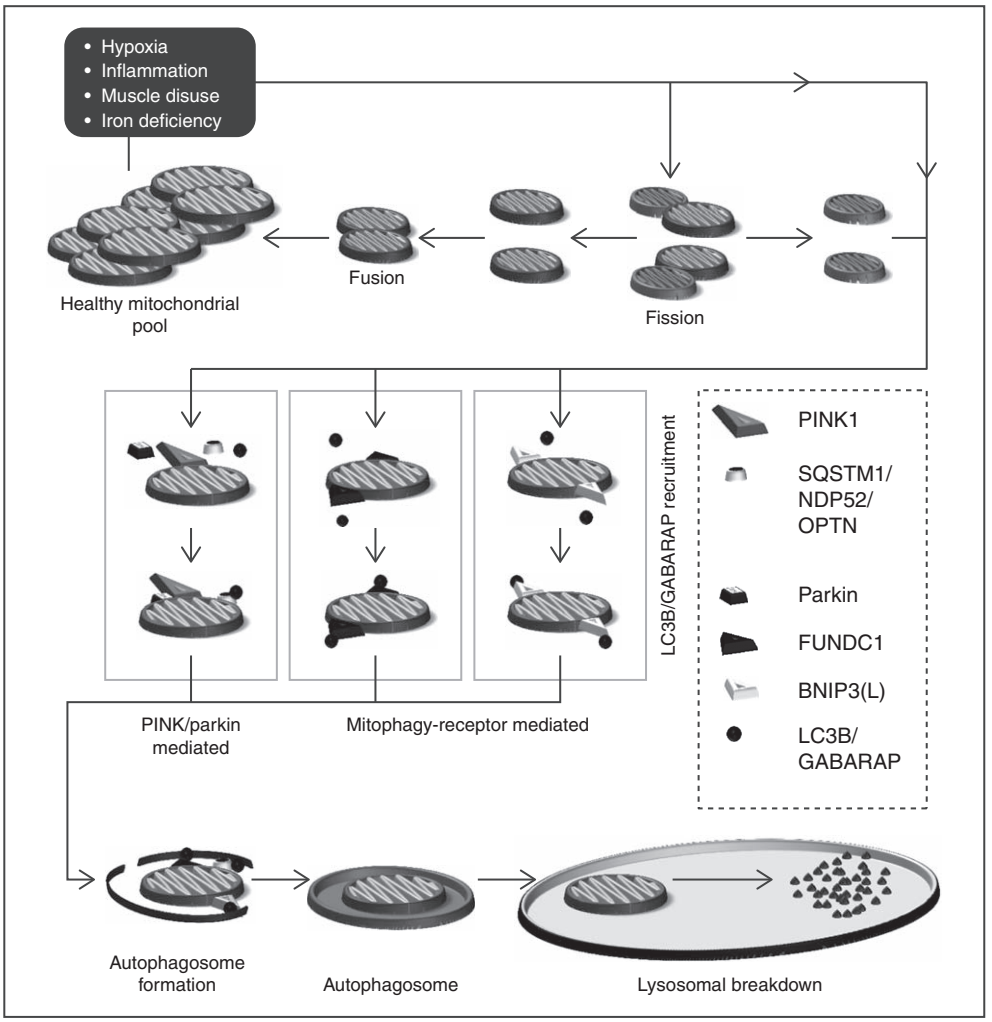

FIGURE 1. Proposed mitophagy-initiation pathway in skeletal muscle of patients with chronic disease. Skeletal muscle is exposed to hypoxia, inflammation, muscle disuse, and/or iron deficiency, resulting in either mitochondrial damage or simply the need to adjust or recycle healthy mitochondrial content. Mitochondrial fission is initiated depending on mitochondrial size and the extent of mitochondrial damage, leaving either parts of or the complete mitochondria for breakdown. Subsequently, the mitochondria are primed for autophagosomal engulfment by LC3B/GABARAP recruitment. LC3B/GABARAP either binds the activated receptor proteins BNIP3, BNIP3L, or FUNDC1, or in case of dysfunctional mitochondria to PINK1-recruited Parkin/SQSTM1, NDFP52, or OPTN. LC3B/GABARAP mediates autophagosomal formation around the mitochondrion and subsequent lysosomal breakdown.

associated protein 1A/1B-light chain 3 (LC3) or γ-aminobutyric acid receptor-associated protein (GABARAP) family is facilitated, and subsequent autophagosomal engulfment and lysosomal degradation is initiated. The selection of mitochondria which are targeted for mitophagy via this pathway is based on the activation of these receptors and not directly related to mitochondrial health [14,17,18].

The second mitophagy pathway is the phosphatase and tensin homologue-induced kinase 1 (PINK1)/E3 ubiquitin-protein ligase parkin (Parkin) pathway, which is specific for mitochondria with a dysfunctional membrane potential [16▪]. In healthy mitochondria, PINK1 is continuously imported to the IMM space where it is broken down by mitochondrial proteases and the proteasome. When the mitochondrial membrane potential is lost however, PINK1 import is compromised and it accumulates on the OMM of the mitochondria. There it attracts and activates several proteins of which Parkin has been described best, but nuclear dot protein 52 kDa (NDP52) and optineurin (OPTN) were found to be recruited independently of Parkin as well [19▪▪,20]. Activated Parkin subsequently ubiquitinates several OMM bound proteins, which can be used as docking station for sequestosome 1 (SQSTM1). SQSTM1,

OPTN, and NDP52 are autophagy receptors which, like the mitophagy receptor proteins discussed above, are able to bind a member of the autophagosomal-specific protein family, leading to subsequent autophagosomal engulfment and lysosomal degradation [14,16▪,17].

EVIDENCE FOR SKELETAL MUSCLE MITOPHAGY IN CHRONIC DISEASE

To date, only a selected number of studies have investigated mitophagy in the skeletal muscle of patients with COPD, T2D, or CHF. Guo *et al.* [21▪] reported increased presence of the mitophagy-related proteins BNIP3 and PARK2 together with an upregulation of general autophagy in *vastus lateralis* muscle of COPD patients. However, whether this was indeed associated with altered mitochondrial content or oxidative capacity was not assessed in this study [21▪]. In contrast, Kruse *et al.* [22] reported no differences in autophagy-related and mitophagy-related markers in the *vastus lateralis* muscle type 2 diabetic study participants compared with healthy volunteers. However, as no markers for oxidative capacity or mitochondrial quantity were reported, oxidative capacity loss in this study population remains undetermined [22].

As mentioned earlier, fission is often associated with mitophagy and could therefore be regarded as an indirect indicator of mitophagy. Average mitochondrial size was shown to be decreased in *vastus lateralis* muscle of patients with CHF [23] or T2D [24], which is suggestive of a mitochondrial fission/fusion balance change toward mitochondrial fission. However, decreased mitochondrial size was not found in COPD patients [25]. Bach *et al.* [26] reported decreased MFN-2 gene expression in the skeletal muscle of T2D patients, whereas Garnier *et al.* [27] reported no significant differences in skeletal muscle MFN-2 gene expression in CHF patients compared with sedentary controls. Despite this unchanged gene expression, Molina *et al.* [4] reported that the skeletal muscle protein expression of MFN-2 was decreased in CHF patients compared with sedentary controls. Taken together, these data point toward enhanced muscle mitophagy in chronic diseases.

TRIGGERS OF MITOPHAGY IN CHRONIC DISEASE

Disease-related factors already known for their ability to impair muscle oxidative capacity and hence putative mitophagy initiators include hypoxia, systemic inflammation, muscle inactivity, and iron deficiency [1,3,5,28,29].

Hypoxia

As recently reviewed, hypoxia was consistently shown to result in decreased skeletal muscle mitochondrial density and oxidative capacity, in both rodents and humans [30]. Increased BNIP3-mediated mitophagy was found during hypoxia in several nonmuscle in-vitro cell lines [31], and increases in BNIP3 gene expression were reported in both murine skeletal muscle tissue and in-vitro cells exposed to hypoxia [32▪]. Transcriptional initiation of mitophagy receptors BNIP3 and BNIP3L by hypoxia is the result of increased ROS production, and subsequent hypoxia-inducible factor 1-α (HIF-1α) stabilization [33,34▪].

In addition to BNIP3(L)-mediated mitophagy, activation of mitophagy receptor FUNDC1 was reported during hypoxia. Interestingly, hypoxia does not seem to affect FUNDC1 transcription, which is in contrast with hypoxia-induced BNIP3(L) transcription, but activates FUNDC1 on a post-transcriptional level by phosphorylation or inhibition of dephosphorylation [35,36▪,37]. Whether crosstalk between the BNIP3(L) and FUNDC1 pathways is present during hypoxia-induced mitophagy remains to be studied.

Although it is thought that human skeletal muscle is quite resistant to chronic hypoxia-induced HIF-1α stabilization, HIF-1α-specific muscle adaptions have been reported [38]. For mitophagy-specific signaling, however, a study comparing gene expression of BNIP3, GABARAPL, LC3, and Beclin in the *vastus lateralis* of hypoxemic COPD patients to normoxemic COPD patients reported no differences [39], although this study only reported gene expression and only for a limited number of mitophagy-related proteins. To date, it remains to be determined whether chronic disease-induced skeletal muscle hypoxia results in mitophagy, and whether this mitophagy targets healthy or dysfunctional mitochondria.

Inflammation

Systemic inflammation results in a decreased skeletal muscle oxidative capacity in mice [40], and skeletal muscle inflammatory signaling is associated with decreased skeletal muscle oxidative phenotype in chronic disease [41]. Like with hypoxia, systemic inflammation is associated with increased ROS production in skeletal muscle mitochondria [42]. However, whether inflammation induced ROS production also results in HIF-1α stabilization and subsequent BNIP3L-mediated mitophagy remains undetermined. ROS also initiates the NF-κB signaling pathway [42], which was indeed shown to causally induce LC3B and

GABARAPL1 gene expression in the skeletal muscle of a systemic inflammation mouse model [43]. However, increased GABARAPL1 gene expression was still found upon NF-κB signaling inhibition, and increased BNIP3 gene expression was found to be completely NF-κB independent in this model [43]. It is therefore assumable that BNIP3-mediated mitophagy is present during systemic inflammation, but the regulatory mechanisms still need to be unraveled.

Interestingly, inflammation-induced mitophagy was found to be regulated through the PINK1/Parkin pathway as well. It has been shown that increased ROS production leads to opening of the mitochondrial permeability transition pore, a large IMM and OMM spanning channel that upon opening disrupts the mitochondrial membrane potential and leads to mitochondrial dysfunction [44]. These dysfunctional mitochondria stabilize PINK1 and subsequently recruit Parkin, making this a pathway that selectively targets dysfunctional mitochondria. Alongside this pathway however, NF-κB was found to be able to stabilize cytosolic PINK1 as well, and is therefore able to target healthy mitochondria for nonselective mitophagy [45▪]. In inflammation subjected cardiac muscle, PINK1/Parkin-mediated mitophagy was indeed found to be increased, but Parkin was found to be dispensable for mitochondrial clearance in this model [46▪]. In line with this, Parkin was found to be unessential in other models as well, as NDP52 and OPTN were identified as alternative receptor-proteins [19▪▪,20]. In conclusion, it is feasible that mitophagy is enhanced in skeletal muscle of patients suffering from (low-grade) systemic inflammation, and is hence underlying loss of oxidative capacity.

Muscle disuse

Physical inactivity and muscle disuse are associated with decreased muscle oxidative capacity [47]. In addition, increased mitophagy was shown in different immobilization studies. Kang *et al.* [48▪] recently showed increased Parkin, decreased BNIP3, and increased general autophagy-related protein expression in unloaded *tibialis anterior* muscle of mice, and Vainshtein *et al.* [49▪] found an increase in BNIP3L, Parkin, LC3BII, and SQSTM1 protein expression in the *tibialis anterior* after denervation. Although no literature is available on mitophagy in human immobilized muscle, the occurrence of mitophagy is feasible as Gram *et al.* [50] found increased ROS production in human immobilized skeletal muscle. In contradiction, Drummond *et al.* [51▪] found that inactive frail women had both decreased BNIP3 and Parkin gene expression in their *vastus lateralis*, which the authors explained as a possible adapted homeostasis to low muscle mass and physical function. Taken together, it is likely that muscle disuse is an important early trigger of mitophagy, probably as part of a normal physiological adaptation to adjust mitochondrial content to the reduced energy demand associated with lower physical activity levels. Whether this mitophagy targets healthy unused mitochondria, dysfunctional mitochondria, or both is unknown.

Iron deficiency

Iron deficiency has been linked to impaired exercise capacity and decreased mitochondrial activity in animal models [28]. In addition, iron deficiency in CHF patients is correlated with impaired exercise capacity [52]. Moreover, iron therapy resulted in increased physical performance in iron-deficient CHF patients [28], suggestive of improved oxidative capacity. Although no studies have been performed to link iron deficiency to increased mitophagy in the skeletal muscle, iron deficiency was shown to induce BNIP3(L) and PINK1/Parkin-mediated mitophagy in *C. elegans* and several nonmuscular human cell-lines [53,54▪▪]. As mitochondria contain high amounts of iron, it could be speculated that mitophagy is required during episodes of iron deficiency to mobilize iron for other essential iron-dependent processes.

MITOPHAGY: FRIEND OR FOE?

The question arises whether skeletal muscle mitophagy is a good or a bad process. On the one hand, mitophagy may serve to clear healthy mitochondria in case they are either redundant or contain essential components required elsewhere. On the other hand, mitophagy is essential for mitochondrial quality control and attenuated mitophagy could result in extensive mitochondrial damage, dysfunction, and even cell death.

Although the knowledge about skeletal muscle mitophagy is still limited, many studies have been performed in heart muscle, which have been extensively reviewed [55▪▪]. Indeed, it has been well established that both insufficient and exacerbated mitophagy in the heart leads to cardiomyopathy, and should, therefore, be tightly regulated [55▪▪]. Such a safeguarding function for mitophagy has also been found in human vascular smooth muscle cells [56]. Interventions to combat the loss of muscle oxidative capacity targeted directly at mitophagy-signaling should, therefore, be approached with the highest caution.

A reduction in mitophagy will not only rescue healthy mitochondria but result in an increased

number of dysfunctional mitochondria as well, which could aggravate the decrease in muscular health even more. Therefore, it is instrumental to obtain additional knowledge concerning the disease-related mitophagy inducing triggers and the mitophagy signaling pathways. Moreover, it could be argued that interventions should be aimed at the prevention of mitochondrial damage, mitochondrial disuse, and nutrient shortage, rather than the pharmacological inhibition of mitophagy pathways itself.

CONCLUSION

The balance between mitophagy and mitochondrial biogenesis is a tightly regulated process, which can be affected by many different factors. We identified several factors which are shared between different chronic diseases, each able to initiate mitophagy independently. We propose that these factors work together as a complex combination of synergistic mitophagy triggers (Fig. 1), resulting in the loss of skeletal muscle oxidative capacity in patients with chronic disease. To prevent the loss of oxidative capacity, it could be argued that therapies should focus on these mitophagy-inducing triggers rather than mitophagy itself, to prevent aggravation of mitochondrial damage and subsequent muscle disorder.

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Conflicts of interest

There are no conflicts of interest.

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