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TOPICAL REVIEW

Perspectives on the interpretation of mitochondrial responses during skeletal muscle disuse-induced atrophy

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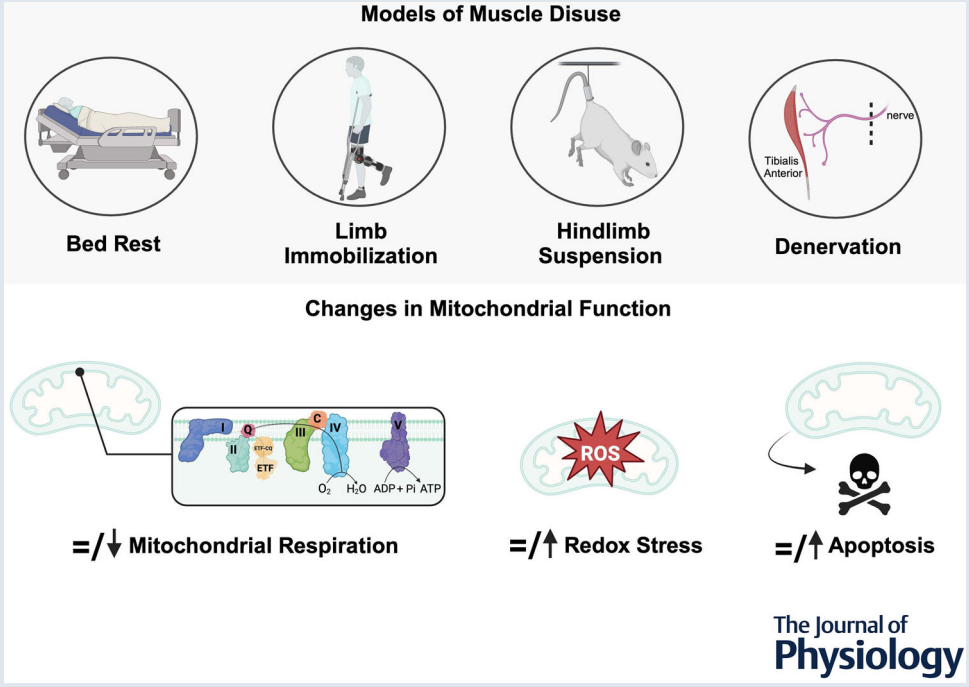

Abstract figure legend Common human and rodent muscle disuse models and associated changes in skeletal muscle mitochondrial function; respiration, redox stress and apoptotic cell death. I–V, complexes I–V; ETF, electron transfer flavoprotein; Q, Q cycle; C, cytochrome *c*; Pi, inorganic phosphate; ROS, reactive oxygen species.

Luca J. Delfinis began his MSc under the supervision of Dr Christopher Perry, investigating time-dependent changes in skeletal muscle mitochondrial function in a subcutaneous C26 colorectal cancer model. Building on these findings, he shifted his focus to an orthotopic ovarian cancer model for his doctoral studies, where he examined the effects of mitochondrial therapeutics on muscle quality and function during cancer progression. **Shahrzad Khajehzadehshoushtar** completed a MSc focused on mitochondrial-mediated cell death pathways in skeletal muscle within the same preclinical orthotopic ovarian cancer model.

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Abstract Reductions in skeletal muscle mitochondrial respiration or increases in mitochondrial reactive oxygen species (ROS) are often interpreted as 'mitochondrial dysfunctions'. However, such changes can also occur as intentional programmed responses to stressors. The term 'mitochondrial dysfunction' could therefore consider the net impact of such responses on other cellular functions. In the case of disuse-induced skeletal muscle atrophy, lower mitochondrial respiration, increased ROS and increased mitochondrial-linked apoptosis have been associated with muscle loss. Such observations support hypotheses that mitochondria contribute to atrophy. If true, there are exciting opportunities for exploring therapeutic strategies that prevent such changes in mitochondrial metabolism. These observations might also support alternative hypotheses where mitochondria are intentionally reprogrammed to serve specific purposes, such as a recalibration of ATP supply to reduced ATP demand during disuse. The goal of this review is to describe what is known regarding skeletal muscle mitochondrial functional responses to muscle disuse, as well as to discuss how these foundational discoveries might lead to new directions that determine whether mitochondrial responses to disuse are causal of atrophy or are adaptive in nature. Three critical questions for consideration include: (1) when is a change in mitochondrial function 'dysfunctional'; (2) how might changes in mitochondrial function represent intentional reprogramming to serve specific purposes; and (3) what factors should be considered when constructing experimental designs to determine the role of mitochondrial functional responses to disuse? Understanding when mitochondrial functional remodelling are dysfunctions or adaptive responses could inform new therapeutic approaches to maintain muscle mass during periods of disuse.

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Introduction

Muscle atrophy, defined as a reduction in muscle mass, can occur in periods of disuse such as limb immobilization during rehabilitation of bone fractures or soft tissue injuries, chronic bed rest during illness or injury, sedentarism, or, in the case of respiratory muscles, mechanical ventilation, amongst other situations. Muscle atrophy can be the result of an imbalance between protein synthesis and degradation with the latter mediated by the ubiquitin-proteasome system (UPS), although some evidence suggests heightened autophagic-lysosomal removal of cellular structures may also contribute (Bonaldo & Sandri, 2013). Atrophy is linked to loss of strength, physical disability and even mortality (Baumgartner et al., 1998; Goodpaster et al., 2006; Janssen et al., 2002). Although the causes of atrophy during disuse are undoubtedly multifactorial, mitochondrial contributions have also been linked to this decline(Hyatt et al., 2019). Indeed, lower mitochondrial respiration and elevated mitochondrial reactive oxygen species (ROS) measured *in vitro* have been reported in several forms of inactivity-induced muscle atrophy (Kavazis et al., 2009; Muller et al., 2007; Picard et al., 2012; Yajid et al., 1998). This relationship suggests that mitochondrial dysfunction may somehow contribute to muscle atrophy. There is also opportunity to consider alternative possibilities where changes in mitochondrial functions are a stress response intended to counter the detrimental effects of atrophy, or simply a consequence of reduced demand for mitochondria. Indeed, endurance exercise training increases mitochondrial content and measured rates of respiration *in vitro* but these changes are lost after several weeks of detraining. It is uncommon to view this withdrawal of mitochondrial adaptations as a mitochondrial 'dysfunction' during detraining. Rather, this example demonstrates a theoretical system that matches supply (mitochondrial capacities) to demand (chronic levels of activity).

In addition to measures of oxidative phosphorylation that are usually performed with assessments of mitochondrial oxygen consumption or 'respiration', some studies have examined how mitochondrial ROS are altered by disuse whereas others have included measures of mitochondrial-induced apoptosis through formation of the mitochondrial permeability transition pore (mPTP). Changes in these measures during disuse atrophy intuitively suggest mitochondrial 'dysfunctions', but they might also be considered more broadly as 'stress responses' if it is assumed that disuse is a cellular

stress (Fig. 1). With this perspective, a consideration for future research is whether ROS serve as essential signals to activate stress programs that might temper or limit the atrophy process consistent with the known roles of mitochondrial ROS as positive regulators of adaptive programs in other models (Sies & Jones, 2020), and also whether mitochondrial permeability transition (mPT) has no bearing on muscle fibres.

Following a brief overview of mitochondrial ATP synthesis, ROS production and mPTP formation, a review of the literature is provided with regards to how these functions change in response to disuse. Finally, different perspectives are discussed with respect to how this foundational literature can be used to further explore whether mitochondrial stress responses cause atrophy, reflect adaptions to the disuse or simply follow a law of demand and supply with regards to energy homeostasis.

Brief overview of select mitochondrial bioenergetic functions

Assessing mitochondrial ATP synthesis with techniques in mitochondrial respiration. Energy is transmitted from glucose, fatty acids and amino acids in our diet to mitochondria in the form of electrons and protons. Briefly, embedded in the inner membrane are protein complexes designed to shuttle electrons (complexes I–IV, coenzyme Q and cytochrome *c*) popularly termed the electron transport chain (ETC) (Nicholls & Ferguson, 2013). The two electron carriers – NAD^+ (i.e. nicotinamide adenine dinucleotide) and FAD (i.e. flavin adenine dinucleotide) – harvest electrons from carbohydrates through glycolysis, fatty acids through beta-oxidation and certain amino acids to ultimately form NADH and $FADH_2$. Electrons held in these reduced carriers are transferred across the ETC through an increasing series of redox potentials with oxygen being the strongest

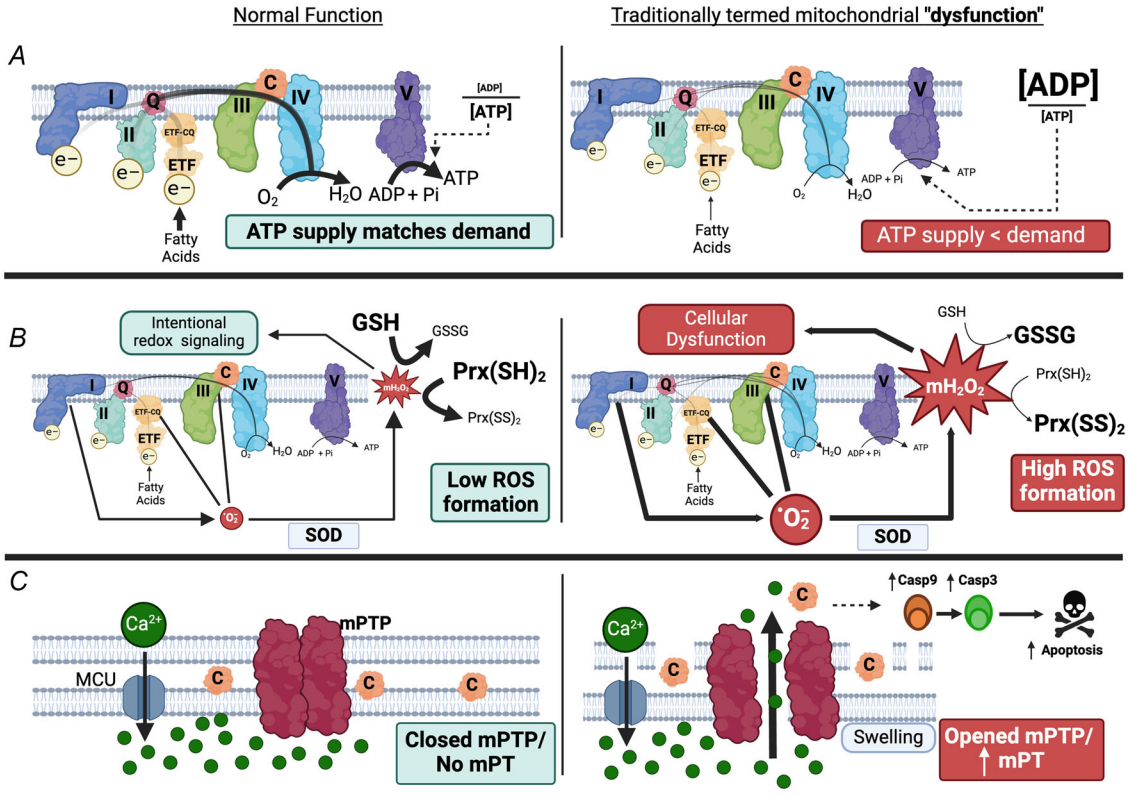

Figure 1. Mitochondrial bioenergetic functions in disuse atrophy
Schematic representation of three commonly measured mitochondrial bioenergetic functions in the field of disuse atrophy (left) with examples of mitochondrial stress responses traditionally termed 'dysfunctions' (right). C, cytochrome c; Casp3, Caspase 3; Casp9, caspase 9; e^-, electron; ETF, electron transfer flavoprotein; GSH, reduced glutathione; GSSG, oxidized glutathione; I–V complexes I–V; MCU, mitochondrial calcium uniporter; mH_2O_2, mitochondrial H_2O_2; mPT, mitochondrial permeability transition; mPTP, mitochondrial permeability transition pore; $O_2{\cdot}^-$, superoxide; Pi, inorganic phosphate; $Prx(SH)_2$ reduced pyridoxine; $Prx(SS)_2$ oxidized pyridoxine; Q, Q cycle. Information retrieved from Nicholls & Ferguson 2013; and Seifert et al., 2009 for panel A, Brand et al., 2010; Nicholls & Ferguson 2013; and Sies & Jones 2020 for panel B, and Wrogemann & Pena 1976 and Nikoletopoulou et al., 2013 for panel C.

electron acceptor at complex IV (Nicholls & Ferguson, 2013). Matrix-derived protons (H^+) are pumped through complexes I, III and IV and accumulate within the intermembrane space. The resulting electrochemical gradient across the inner membrane contributes to a membrane potential that determines the proton motive force, which itself drives H^+ re-entry into the matrix through ATP synthase (complex V). This flux couples inorganic phosphate (P_i) to ADP to produce ATP (Mitchell, 1961). This process of *oxidative phosphorylation* of ADP and P_i to ATP represents a considerable source of ATP for skeletal muscle when oxygen is available at sufficient quantities (Nicholls & Ferguson, 2013). The consumption of oxygen at complex IV is termed 'mitochondrial respiration' where measures of mitochondrial oxygen consumption in tissue samples are commonly used as an index of mitochondrial ATP synthesis. Because H^+ can also re-enter the matrix through uncoupling proteins without synthesis of ATP, careful consideration of experimental conditions is required to understand whether changes in respiration are a result of oxidative phosphorylation or uncoupling, although this is outside the scope of the present review. Further information, including the broader concept of how mitochondrial regulation of [ATP], [ADP], [H^+] and [Pi] equilibria is a critical determinant of the free energy of ATP hydrolysis by ATPases and other ATP-dependent proteins in the cytosol, is provided by Nicholls and Ferguson (2013).

In the disuse atrophy literature, mitochondrial oxygen consumption is often measured with the use of 'respirometers', which use oxygen sensors to monitor the rate at which oxygen is removed from an aqueous media containing mitochondria prepared from skeletal muscle biopsies. 'Mitochondrial respiration' is a term used to interpret this measurement. Respiration is stimulated by adding substrates capable of producing NADH or $FADH_2$ in media that contains, in part, Pi and ADP to support oxidative phosphorylation. In this way, *in vitro* assessments of mitochondrial respiration can be interpreted within the context of the substrate used in the experiment. It is common to use pyruvate as an index of glucose oxidation given that the preparation of mitochondrial samples often removes the cytoplasm and parts of the glycolytic pathway such that use of glucose is limited in these *in vitro* assays. Likewise, fatty acids in various forms are often used to stimulate respiration as an index of mitochondrial fat oxidation, wheras amino acid-linked substrates such as glutamate (derived in part from glutamine) are typically used to assess amino acid oxidation. As pyruvate and glutamate produce NADH through pyruvate dehydrogenase (PDH) and glutamate dehydrogenase (GDH), respectively, these substrates also provide insight into the activity of their respective dehydrogenases as well as complex I-stimulated respiration. Fatty acids produce NADH as well as $FADH_2$ through beta oxidation and the tricarboxylic acid cycle, with the latter two supporting respiration through the electron transfer flavoprotein (ETF) (Seifert et al., 2009) and complex II, respectively (Nicholls & Ferguson, 2013). As such, decreases in respiration observed in a given investigation may not be reflective of all fuel selection pathways and must be interpreted within the context of the specific substrate used to stimulate respiration. More, extensive overview of substrate titrations, including protocols that stimulate other sites of electron entry into the ETC such as complex II and glycerol-3-phosphate dehydrogenase, are provided elsewhere (Mráček et al., 2013; Pesta & Gnaiger, 2012).

Reductions in substrate-specific mitochondrial respiration as a 'dysfunction' could instead represent adaptive responses related to reduced supply to match lower metabolic demand as would occur during disuse (Fig. 2, top). Alternatively, there could be changes in fuel selection where reports of lower mitochondrial respiration in response to one substrate may not be true for another substrate. The degree to which this change represents a 'dysfunction' or a metabolic reprogramming to serve another purpose, such as redirecting substrates to non-energy fates, becomes uncertain. With this perspective, a measurement of mitochondrial respiration with only one of glucose, fatty acid or amino acid-derived substrates may not provide sufficient information to define 'dysfunction' unless comparisons are made between substrates and if controlled investigations that determine whether reductions in substrate-specific respiration are limiting ATP supply to ATP-dependent processes during contraction. The link to atrophy becomes less clear with regards to reduced mitochondrial ATP supply. This notion is discussed in more detail at the end of this review.

ROS formation. A natural by-product of oxidative phosphorylation is the generation of ROS. If electrons slip prematurely from complex I or III before they reduce oxygen at complex IV, they form a superoxide anion ($O_2^{\bullet -}$) radical (Brand, 2010; Nicholls & Ferguson, 2013). Electrons can also slip prematurely through the ETF or other dehydrogenases including PDH and throughout the tricarboxylic acid cycle to generate superoxide. While $O_2^{\bullet -}$ is reactive, it is quickly degraded by superoxide dismutase (SOD) to hydrogen peroxide (H_2O_2) (Halliwell, 2006). At low rates of production, H_2O_2 is critical for cell signalling events to activate proliferation, differentiation, migration and angiogenesis (Sies & Jones, 2020). However, at high rates of production, H_2O_2 can trigger growth arrest and cell death (Sies & Jones, 2020). The topic becomes more complex and opportunistic for insight when considering factors beyond concentration or rates of production, including how H_2O_2 from mitochondria can be directed to target proteins in other compartments

such as the cytoplasm or adjacent organelles such as the sarcoplasmic reticulum, which regulates calcium cycling.

ETC-derived superoxide generation, and hence H_2O_2 production, is favoured when mitochondrial membrane potential is high as occurs when reducing equivalent supply exceeds the relative demand set by the ADP/ATP equilibria (and P_i) (Nicholls & Ferguson, 2013). In other words, this theory predicts that mitochondrial superoxide production is higher when metabolic demand is lower, which prompts consideration of the relationship between physical inactivity, ADP/ATP turnover and mitochondrial H_2O_2 emission. H_2O_2 is scavenged by glutathione as well as peroxiredoxins such that observations of increased H_2O_2 will reflect either an increase in its production or decreases in its scavenging. Comparisons of H_2O_2 with the activity of these redox buffering systems can therefore add further insight into the mechanisms of mitochondrial ROS and their influence in the muscle cell.

Traditionally, increases in H_2O_2 are interpreted as a mitochondrial 'dysfunction' because it is often assumed that this will lead to a cellular dysfunction, such as atrophy. Although this is possible, the measurement on its own does not rule out the possibility that H_2O_2 serves as a redox signal to upregulate adaptive pathways that attenuate the rate of atrophy. H_2O_2 triggers adaptive responses in other models (Sies & Jones, 2020), which highlights the potential for designing new studies that relate H_2O_2 to redox-sensitive proteins with known roles in regulating muscle mass or quality (Fig. 2, middle).

mPT through formation of mPTP. Mitochondria can trigger apoptosis by experiencing mPT through formation of the mPTP. Although the sarcoplasmic reticulum is the main organelle responsible for buffering Ca^{2+}, the mitochondria are also capable of taking up this ion, especially during times of cytosolic Ca^{2+} overload. Excess Ca^{2+} into the cell can trigger the opening of the mPTP, which can then activate apoptotic cell death pathways (Rasola & Bernardi, 2011; Wrogemann & Pena, 1976). By convention, mPT refers to the measurement of the event itself, whereas mPTP refers to assessments of the structure of the pore itself. In the disuse literature, all reported measures to date pertained to mPT and were performed by assessing *in vitro* the amount of calcium that mitochondria require to undergo permeability transition. This 'mitochondrial calcium retention capacity' (CRC)

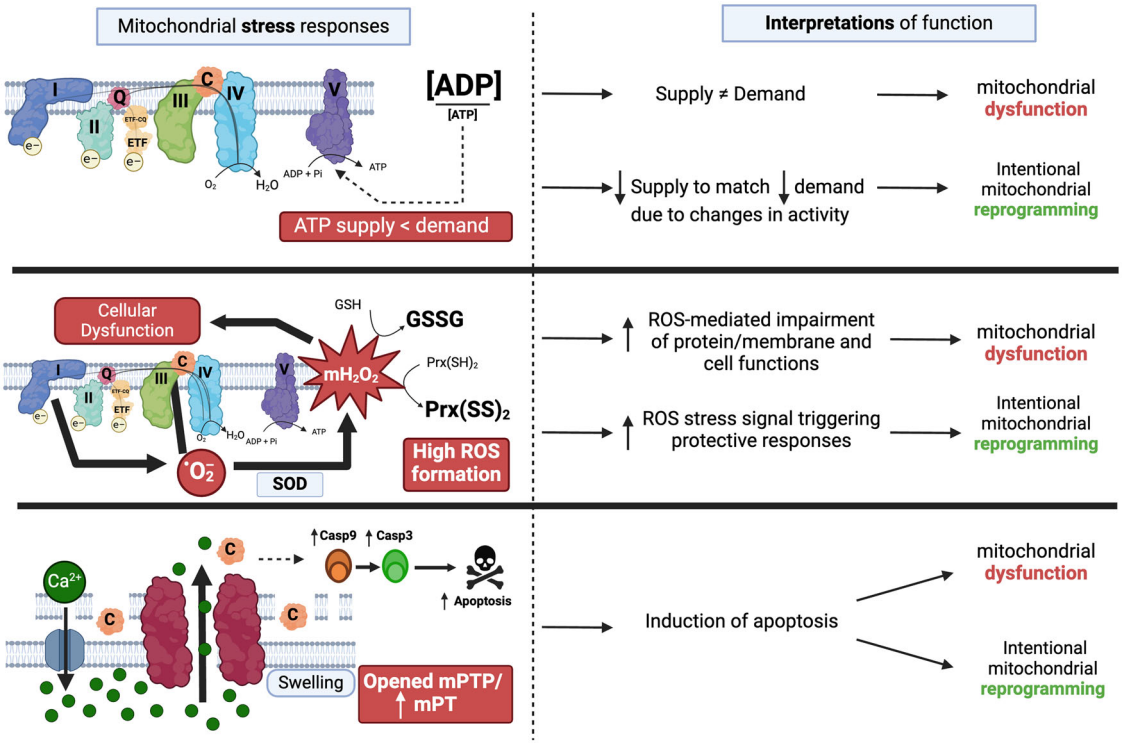

Figure 2. Mitochondrial stress responses reflecting dysfunction or intentional adaptation
Schematic representation of mitochondrial stress responses that could reflect a dysfunction or intentional adaptation. C, cytochrome c; Ca^{2+}, calcium; Casp3, Caspase 3; Casp9, caspase 9; e^-, electron; ETF, electron transfer flavoprotein; GSH, reduced glutathione; GSSG, oxidized glutathione; I–V complexes I–V; MCU, mitochondrial calcium uniporter; mH_2O_2, mitochondrial H_2O_2; mPT mitochondrial permeability transition; mPTP, mitochondrial permeability transition pore; $O_2^{\cdot -}$, superoxide; Pi, inorganic phosphate; $Prx(SH)_2$ reduced pyridoxine; $Prx(SS)_2$ oxidized pyridoxine; Q, Q cycle.

measurement can therefore be related to downstream apoptotic pathways linked to mitochondrial calcium overload. Specifically, mPT promotes the release of cytochrome *c*, which binds APAf-1, forming an apoptosome that can cleave caspase-9 and -3 to trigger apoptosis (Nikoletopoulou et al., 2013) (Fig. 2, bottom).

The outer mitochondrial membrane-bound pro-apoptotic proteins Bak and Bax and anti-apoptotic protein Bcl2 have also been implicated in disuse atrophy as discussed below, although their role with regards to the mPTP remains unclear. As reviewed elsewhere, they may be activated in response to swelling that is linked to mPTP (Bernardi et al., 2022).

In the disuse atrophy literature, the degree to which apoptosis contributes to the death of an entire muscle fibre remains unknown and controversial. It is also not clear whether apoptosis can be used to regulate remodelling of specific regions of muscle fibres. Furthermore, apoptosis is understood as 'programmed' cell death in other cell types becais this process removes harmful cells, maintains tissue homeostasis and aids in immune regulation (Ellis et al., 1991). Therefore, triggering apoptosis may be beneficial in some contexts depending on cellular demand. It is difficult to reconcile this notion in skeletal muscle fibres, and the field requires much research in this regard. Nonetheless, these perspectives shape how measurements of CRC can be used to form new questions, particularly in the context of calcium stress and the potential for this process being a form of 'programmed' destruction that might avoid worse outcomes such as necrotic cell death, which can often lead to fibrosis.

Activation of caspase-3 as a result of mPT has been shown to interact with the UPS, a key mechanism responsible for degrading most cytoplasmic and nuclear proteins during apoptosis (Thibaudeau & Smith, 2019). Briefly, the UPS functions by attaching ubiquitin moieties to target proteins, effectively 'marking' them for degradation by the 26S proteasome (Thibaudeau & Smith, 2019). The 26S proteasome consists of the 19S regulatory subunit(s), which recognize ubiquitinated proteins, and the 20S proteasome, which carries out protein degradation. Wang et al. (2010) demonstrated that caspase-3 interacts with specific subunits (Rpt2 and Rpt6) of the 19S regulatory complex, enhancing their sensitivity and leading to increased proteasome activity in C2C12 myotubes. Similarly, in a murine model of chronic kidney disease, characterized by accelerated muscle protein degradation and elevated caspase-3 activity, proteasome activity was increased through modifications of the 19S regulatory subunit (Wang et al., 2010).

Earlier work by Du et al. (2004) in L6 skeletal muscle cells further showed that caspase-3 directly cleaves actin and myosin, generating smaller fragments that are subsequently marked for degradation by the UPS, increasing substrate availability for the UPS. Collectively, these findings suggest that mPT-induced caspase-3 activation promotes protein degradation through both non-apoptotic mechanisms, enhancing UPS activity, and apoptotic mechanisms, by directly cleaving proteins and providing additional substrates for the UPS. However, the degree to which proteasomal activity might be regulated by mitochondrial permeability transition in the absence of apoptosis *per se* remains unknown.

Although UPS-mediated protein degradation and increased substrate availability are known contributors to muscle atrophy, this pathway has not been extensively studied in models of muscle disuse. In a denervation study by Plant et al. (2009), caspase-3 knockout mice showed no significant differences in total protein ubiquitination or proteasome activity compared to wild-type controls. These findings suggest that activation of the mPT–caspase-3–UPS axis in muscle disuse remains unclear and warrants further investigation, especially in relation to mitochondrial permeability transition.

Muscle disuse-induced mitochondrial adaptations in non-disease rodent and human models

Hindlimb suspension/unloading-induced-disuse (rodent). Hindlimb suspension is a popular rodent model of muscle disuse (Morey-Holton & Globus, 2002). Several studies have used this model to understand the relationship of disuse, muscle atrophy and resulting impact on markers of mitochondrial content or function (Cannavino et al., 2015; Rosa-Caldwell et al., 2020; Trevino et al., 2019; Wagatsuma et al., 2011; Yajid et al., 1998)

Four weeks of hindlimb suspension in rats decreased hindlimb muscle wet weights (Yajid et al., 1998) in association with lower complex I (pyruvate + malate, NADH), but not complex II (succinate, $FADH_2$), supported mitochondrial respiration in the gastrocnemius despite no such mitochondrial changes in the extensor digitorum longus (EDL), soleus (SOL) and tibialis anterior (TA) (Yajid et al., 1998). However, data from hindlimb suspension in mice demonstrate that mitochondrial stress responses occur even earlier at 3, 7 and 14 days and can exhibit different mitochondrial responses compared to 4 weeks in rats (Trevino et al., 2019; Yajid et al., 1998). After 7 days, SOL muscle wet weight decreases, which is indicative of atrophy (Trevino et al., 2019). However, decreases in complex I- and II-supported respiration (glutamate + malate, NADH; succinate, $FADH_2$ respectively) and calcium retention capacity (increased propensity for mPT) occur as early as 3 days after hindlimb suspension, with mitochondrial H_2O_2 emission increased by 7 days (Trevino et al., 2019). It is important to note that changes in mitochondrial functions were not driven by reductions in complex I–V protein contents given these markers were unchanged

up to day 14 (Trevino et al., 2019). However, others have identified plantarflexion muscle force production decreases as late as 14 days after hindlimb suspension in mice (Oliveira et al., 2019). These studies support further exploration into whether such reductions in respiration led to higher [ADP]/[ATP], which would be reflective of the cell's ability to maintain energy homeostasis. Likewise, exploring whether changes in the oxidation of one substrate (e.g. pyruvate) reflect changes in the oxidation of other substrates (e.g. fatty acids) could lead to new questions focused on altered substrate selection that reflect a need for directing substrates to non-energy fates such as growth or repair, as explored in other disciplines outside of muscle physiology (Stine et al., 2022).

Of interest, there is evidence in rats to suggest that apoptosis is increased in the hindlimb suspension model through measures of mitochondrial-linked caspase 3 activity, Bax, Bcl-2, Apaf-1 and apoptosis inducing factor (AIF) (Leeuwenburgh et al., 2005; Siu et al., 2005). Considering that lower respiration and increased mPT occur as early as 3 days after hindlimb suspension in mice, this could suggest that mitochondria may play an early role in signalling apoptosis in inactive muscle fibres (Trevino et al., 2019). Additionally, an *in vitro* model of disuse atrophy using cultured single muscle fibres from mice demonstrated that inhibitors of mPT, mitochondrial ROS and caspase-3 prevent atrophy (Skinner et al., 2021). An intuitive interpretation is that this induction of apoptosis is a mitochondrial 'dysfunction' by contributing to a loss of fibres and muscle mass, as reviewed elsewhere (Hyatt et al., 2019). Other cell types are known to undergo apoptosis through an intentional 'program' when the cell is no longer needed. The degree to which this occurs in muscle as a 'quality control' mechanism is relatively unexplored but could be considered in the context of ensuring dysfunctional fibres are removed from the muscle, albeit with a net deficit of mass.

Denervation-induced-disuse (rodent). Surgical denervation is another popular model of disuse within rodents. Briefly, the common peroneal nerve in one limb can be excised to denervate the TA and EDL muscles, at the same time as leaving the opposite limb intact to serve as a control limb (Adhihetty et al., 2007; Eisenberg & Hood, 1994; Wicks & Hood, 1991). This renders one limb inactive, forcing the contralateral limb responsible for completing all locomotion.

Reduced muscle wet weights were observed 5 days after denervation and continued to decline up to 21–42 days depending on the muscle group (Adhihetty et al., 2007). Prior to muscle loss, complex I-supported respiration (glutamate, NADH, ADP) in the TA muscle was lower as early as 7 days post-denervation within the subsarcolemmal (SS) mitochondrial pool (Adhihetty et al., 2007). This decrement coincided with reduced cytochrome *c* and COX activity. Maximal mitochondrial ROS production (glutamate, NADH, no ADP) was elevated in the SS pool at 7, 14 and 21 days post-denervation, whereas there were no changes for ROS production in the intermyofibrillar (IMF) mitochondrial pool (Adhihetty et al., 2007). Of interest, denervation has been shown to decrease force producing capabilities as early as 8 days post-denervation within the TA (Wicks & Hood, 1991). Accordingly, the decrease in respiration and mitochondrial enzyme activity, along with heightened ROS production observed by (Adhihetty et al., 2007), may coincide with muscle weakness.

Adhihetty et al. (2007) also identified early pre-atrophy increases in apoptotic signalling (Adhihetty et al., 2007) in as little as 5–7 days after denervation including an increased Bax-to-Bcl-2 ratio and AIF, which were followed by increased calcium overload-induced mPT and a delayed increase in mitochondrial ROS by day 14 using a superoxide-sensitive fluorophore. This suggests denervation-induced disuse initiates early mitochondrial stress responses that may trigger apoptosis early during the process of muscle loss.

The degree to which these mechanisms are linked to weakness independent of atrophy, which may occur prior to atrophy, remains ripe for future investigation. Indeed, unravelling the contributions of each mitochondrial function to atrophy independent weakness or atrophy itself is an opportunity for considerable research.

Limb immobilization-induced-disuse (human). Limb immobilization by cast or brace is a common approach to immobilize the lower limbs and model disuse in uninjured adults. Typically, participants wear a knee brace that fixes the knee at a 60° angle for up to 2 weeks to induce muscle inactivity (Abadi et al., 2009; Edwards et al., 2020; Gram et al., 2015; Hafen et al., 2019; Miotto et al., 2019; Pileggi et al., 2018). A recent meta-analysis highlights how this model is effective at lowering muscle strength and mass, albeit with greater decreases in strength than mass (Preobrazenski, Seigel, et al., 2023). The model also causes reductions in strength and mass in the immobilized limb without appreciable effects on the non-immobilized limb, allowing comparisons between both limbs in the same individual (Preobrazenski, Janssen, et al., 2023). After immobilization, muscle biopsies are often sampled from the vastus lateralis and used to measure different mitochondrial functions.

Several studies evaluated different mitochondrial functions after 14 days of leg immobilization because muscle atrophy and muscle weakness occur within the quadriceps of men and women after this length of time (Abadi et al., 2009). However, muscle atrophy and weakness can also be seen as early as 7 days post cast

immobilization (Edwards et al., 2020). To our knowledge, only one study has evaluated mitochondrial function as early as 3 days post limb immobilization (Miotto et al., 2019) but without measures of muscle mass or size, where complex-I and -II (pyruvate + malate or glutamate, NADH; succinate, $FADH_2$, respectively) supported respiration was decreased concurrently without changes in fatty acid-supported respiration or mitochondrial protein content markers. These findings indicate that reductions in mitochondrial respiration with certain substrates do not reflect a global decrement in respiratory function across all substrates. Specifically, this raises new questions regarding the reason why immobilization might lower oxidation of glucose-derived substrate (pyruvate) but not fatty acids, and guides new perspectives on what defines a 'mitochondrial dysfunction' in this regard. The lack of changes in mitochondrial content markers also points to possibilities of post-translational regulation that could be further explored with regards to pyruvate oxidation (Miotto et al., 2019). Also, although this investigation by Miotto et al. (2019) was part of a larger parent study that demonstrated reduced quadriceps volume by 14 days of immobilization (McGlory et al., 2019), direct comparisons of mitochondrial respiration to muscle mass or size at this very early time point of 3 days would add further insight into whether such mitochondrial changes are a defining pre-atrophy signature in muscle. Indeed, other conditions of muscle wasting show reductions in mitochondrial respiration prior to atrophy such as cancer cachexia (Brown et al., 2017; Delfinis et al., 2022, 2024). Because this study was performed in young adult women, the results inspire new directions to compare biological sexes across the lifespan.

A separate study examined the effects of 7 days of immobilization in young men on skeletal muscle mitochondrial responses and found no effect on complex I or II-supported respiration (pyruvate + malate + glutamate, NADH; succinate, $FADH_2$, respectively) (Edwards et al., 2020). These findings raise questions about potential divergent responses of mitochondria across time during limb immobilization, as well as biological sex differences in the regulation of mitochondrial bioenergetics during disuse, which appears plausible given the increasing awareness that mitochondrial responses to various disorders can differ between sexes (Junker et al., 2022). To our knowledge, there are no data investigating the relationship of mPT and apoptosis between 3 and 7 days of limb immobilization.

Mitochondrial alterations are observed before muscle atrophy, emerging after 3 days of immobilization-induced muscle disuse. However, after 10–14 days of limb immobilization, mitochondrial respiratory responses become further complex because muscle weakness, atrophy and mitochondrial alterations appear to occur concurrently. Decreased complex I-supported (pyruvate + malate, NADH) mitochondrial respiration after 14 days of limb immobilization was reported in young and older men (Gram et al., 2015) and young women (Miotto et al., 2019), whereas no changes occurred in middle aged men (Pileggi et al., 2018). These differing results are difficult to explain given that not all studies tracked changes in mitochondrial content to explain the respiration responses.

The relationships between limb immobilization-induced disuse and mitochondrial energetics become further complex in the context of mitochondrial H_2O_2. Mitochondrial H_2O_2 emission was unaltered 3 days post-limb immobilization, whereas there were no changes in protein content of certain antioxidant enzymes found in the cytoplasm and mitochondria (Miotto et al., 2019). This suggests metabolic changes occur prior to redox stress responses in this model. After 14 days, mitochondrial H_2O_2 emission were increased in males assessed by Gram et al. (2015) and Pileggi et al. (2018), whereas this measure was unchanged in women in the study by Miotto et al. (2019). However, Gram et al. (2015) and Pileggi et al. (2018) also found that certain cytosolic and mitochondrial antioxidant protein contents were unchanged, whereas Miotto et al. (2019) demonstrate an increase in catalase and SOD2 content. This evidence warrants further examination into the effects of biological sex on mitochondrial redox responses to limb immobilization. Furthermore, the degree to which these changes in mitochondrial H_2O_2 emission trigger atrophy processes remain unclear. To our knowledge, no studies have evaluated mPT and mitochondrial-linked apoptosis after 14 days of limb immobilization.

Bed rest-induced-disuse (human). Bed rest is another popular model of disuse given many diseases or injuries can lead to atrophy after prolonged bed rest. Using this model, several studies have examined the skeletal muscle mitochondrial responses in healthy uninjured young or older adults across a period of 4–55 days (Dirks et al., 2020; Dulac et al., 2024; Eggelbusch et al., 2024; Kenny et al., 2017; Larsen et al., 2018; Salvadego et al., 2016, 2018; Zuccarelli et al., 2021). Reductions in lean mass have been reported at 15–21 days following bed rest (Eggelbusch et al., 2024; Kenny et al., 2017; Salvadego et al., 2016), with one study reporting whole body lean mass reductions at 10 days with no change in lower limb lean mass (Zuccarelli et al., 2021), while more robust loss was seen at 60 days (Eggelbusch et al., 2024). However, these studies did not use histological measures to assess cross-sectional area (CSA) of fibres to confirm muscle atrophy.

Four days of bed rest have no effect on complex I and II mitochondrial respiration normalized to wet weight of muscle (Larsen et al., 2018). However, CS

activity was significantly decreased. Interestingly, once mitochondrial respiration is normalized to CS activity as an index of mitochondrial content, respiration was actually greater compared to control levels when assessed with pyruvate, glutamate and succinate but not fatty acids (Larsen et al., 2018). This early mitochondrial response to upregulate oxygen consumption is interesting because it represents a unique energetic response to disuse not yet identified in any of the other models discussed in this review. This point also highlights the value of normalizing respiration to wet weight as well as mitochondrial content markers to determine whether changes in respiration are a result of intrinsic alterations within mitochondria or mitochondrial content itself.

By contrast, 7 days of bed rest decreased maximal ADP-stimulated complex I- (pyruvate + malate, NADH) and II- (succinate, $FADH_2$) supported mitochondrial respiration (Dirks et al., 2020). Intriguingly, after 7 days of bed rest, there are no significant reductions in CS activity, yet, when respiration is normalized to CS activity, there are no longer any statistical differences between control and bed rest participants (Dirks et al., 2020), suggesting variable responses in mitochondrial content markers and respiration. Ten days of bed rest-induced disuse demonstrates similar results, such that there are no changes in complex I- and II- supported respiration (Salvadego et al., 2016). Although there appeared to be no changes in CS activity after 10 days of bed rest (Zuccarelli et al., 2021), normalization of respiration to this marker, as performed in the 7-day bed rest study (Dirks et al., 2020), was not conducted. After 7 and 10 days of bed rest-induced disuse, there are mixed reports of either no change in respiratory sensitivity to sub-maximal concentrations of ADP or increases in ADP sensitivity (Dirks et al., 2020; Zuccarelli et al., 2021). Therefore, although maximal respiratory capacity seems to be unaffected after 7–10 days of bed rest, mitochondrial ADP sensitivity appears to increase, which is a unique adaptation compared to other models of disuse.

Using a 14-day head-down tilt bed rest model in men and women aged 55–65 years, leg muscle (quadriceps) volume was reduced as were mitochondrial content markers and complex I- and II- (glutamate + malate, NADH; succinate, $FADH_2$ respectively) supported respiration (Dulac et al., 2024). However, fatty acid supported respiration was unchanged. Therefore, both bed rest and limb immobilization (Miotto et al., 2019), as discussed in the previous section, do not alter fatty acid respiration assessed *in vitro*, at least at maximal rates, which demonstrates that decreases in respiration are substrate-specific during disuse. After 21 days of bed rest, complex I and II-supported respiration (pyruvate + malate + glutamate, NADH; succinate, $FADH_2$ respectively) is decreased when normalized to muscle wet weight but no differences are found when normalized to CS activity (Kenny et al., 2017). Collectively, the effects of bed rest up to 21 days on mitochondrial respiration varies between studies and shows decreased respiration can be driven by either lower mitochondrial content or intrinsic alterations within mitochondria that require further investigation. Investigations could consider measuring the content and post-translational regulation of rate-limiting enzymes that are relevant to the substrates selected for the assessments, as well as compare glucose *vs.* fat-derived substrate oxidation to understand whether a more programmed metabolic switch occurs. There are limited data on the redox stress responses of bed rest-induced disuse. At 4 days of bed rest, there are no reported changes in maximal or submaximal H_2O_2 emissions (Larsen et al., 2018). This trend remained when H_2O_2 emissions were normalized to CS activity (Larsen et al., 2018). Interestingly, however, there are increases in the cytosolic antioxidant catalase after 4 days of bed rest-induced disuse (Larsen et al., 2018). After 7 days of bed rest, maximal H_2O_2 emission (absence of ADP) increased, with no differences in submaximal H_2O_2 emission (Dirks et al., 2020). This trend also remained when normalized to CS activity (Dirks et al., 2020) suggesting the responses were a result of intrinsic alterations. Indeed, mitochondria were more sensitive to ADP-attenuation of H_2O_2 (Dirks et al., 2020) suggesting a specific alteration in how ADP governs membrane potential-driven electron slip (Nicholls & Ferguson, 2013). There were also no differences in oxidized or reduced glutathione, suggesting that changes in H_2O_2 emission did not affect cellular redox conditions (Dirks et al., 2020). No changes in H_2O_2 emission (no ADP) and mPT were observed following 14 days of head-down tilt bed rest, but markers of autophagy were increased (Dulac et al., 2024). Although no direct measures of apoptosis downstream from mPT were performed, such as caspase 9/3 activities or cleavage products, the lack of change in mPT assessed with calcium stress *in vitro* suggest mitochondrial apoptosis may not occur during bed rest.

Although changes in mitochondrial respiration, ROS and apoptosis are inconsistent in the various models of disuse atrophy, a summary of disuse-induced skeletal muscle mitochondrial alterations that occur in both rodent and humans is provided in Table 1. A more detailed summary of changes in disuse-induced mitochondrial function is provided in Table 2.

Perspectives and future directions

To what degree are the mitochondrial responses in models of disuse contributing to atrophy? With regards to mitochondrial respiration, although a causal relationship is not necessarily proposed in most studies, the decreased respiration might be interpreted as a 'dysfunction'

Table 1. Summary of mitochondrial functional responses in multiple models of non-disease-induced muscle disuse

Models of disuse atrophy	Muscle mass	Respiration	Redox Stress	Mitochondrial-linked apoptosis
Hindlimb suspension	↓	↓	↑	↑
Surgical denervation	↓	↓	↑	↑
Cast immobilization	↓	↓	↑/No change	No data
Bed rest (>21 days)	↓	↓/No change (substrate-dependant)	↑/No change	No change (1–14 days)

that would somehow be linked to atrophy. However, it is difficult to develop a framework where limited mitochondrial ATP supply, if it occurs during disuse, is causing atrophy when considering that the regulation of protein degradation and possible autophagy are not necessarily rate-limited by ATP (Bonaldo & Sandri, 2013), nor are mitochondria the only source of ATP. It could be indicative of a broader dysfunction, but this is often not defined or necessarily directly proposed.

In keeping with this consideration, a recent study reported no correlation between mixed substrate-supported respiration (pyruvate, glutamate, succinate) and myofibrillar protein synthesis rates in young adults when separate analyses were performed on samples collected before bed rest or on samples collected after bed rest (Holwerda et al., 2024). However, correlations between the relative changes in either measure from pre- to post-bed rest were not assessed (Holwerda et al., 2024), which means it is difficult to conclude whether *alterations* in respiration and protein synthesis rates occur in tandem. It is also unclear whether changes in quadriceps CSA are correlated to changes in respiration from pre- to post-bed rest. (Holwerda et al., 2024). The lack of significant correlations using separate analyses on either the pre or post-bed rest biopsies are perhaps challenging to reconcile with the larger parent investigations that reported lower respiration (Holwerda et al., 2024) and lower quadriceps mass (Dirks et al., 2019) between pre- and post-bed rest biopsies. Of interest, the lack of changes in myofibrillar protein synthesis rates seen in the parent investigation using young adults, despite reductions in quadriceps mass (Dirks et al., 2019), contrasts with the lower protein synthesis rates seen in older adults after bed rest by another group (Smeuninx et al., 2025). Additional research is warranted into the effect of age on mitochondrial respiratory relationships to protein synthesis rates and muscle mass changes during bed rest. More broadly, there are limited theoretical bases to mechanistically connect mitochondrial ATP synthesis to muscle wasting processes during bed rest given that imbalances between protein synthesis and degradation may not be limited by ATP derived from mitochondria, and the potential for compensatory increases in ATP supply from other pathways such as glycolysis could occur. Rather, other mitochondrial stress responses such as those discussed throughout this review offer a conceptual basis for exploring mitochondrial-derived signals that could contribute to atrophy during disuse.

Understanding the relevance of altered indices of mitochondrial ATP synthesis to muscle disuse could be informed by theoretical models of skeletal muscle metabolic regulation to highlight how disuse could simply cause a realignment of ATP supply to reduced demand. In skeletal muscle, the matching of mitochondrial ATP supply to ATP demand is tightly regulated through feed-forward and feed-back signalling cascades. In this way, physical activity triggers rapid increases in mitochondrial ATP synthesis which, in part, ensures muscle contraction can continue for extended periods of time. Chronic physical activity, such as endurance-type exercise training, increases the efficiency of this matching between ATP supply and demand where the activation of mitochondrial oxidative phosphorylation is accelerated at the onset of exercise or during transitions from low to high exercise intensities (Hargreaves & Spriet, 2020). This adaptive reprogramming of mitochondria arises, in part, from chronic activation of gene programs encoding mitochondrial proteins that sense stressors arising during exercise and regulate the matching of ATP supply to demand, in addition to potential remodelling of mitochondrial structure and quality (Perry & Hawley, 2018). Central to this design is the concept of matching ATP supply to ATP demand. If there is a chronic demand for mitochondrial ATP synthesis, then the system improves to ensure a greater capacity and/or efficiency of this process to meet this demand. This principle therefore predicts that a withdrawal in demand, as occurs during physical inactivity, would lead to reduced demand-driven signals and activation of oxidative phosphorylation. Through this perspective, declines in mitochondrial respiration observed during disuse atrophy can be considered through an additional perspective relating lower rates of muscle contraction to lower metabolic demand for ATP.

Under periods of low ATP hydrolysis in muscle, the ([ADP][Pi])/[ATP] equilibrium between the cytoplasm and mitochondrial matrix is relatively high, which limits the degree of ATP synthesis at ATP

Table 2. Summary of changes in mitochondrial functions in rodent (hindlimb suspension and denervation) and human (limb immobilization, bed rest, head-down tilt bed rest) disuse models

Model	Reference	Disuse duration	Species, sex and age	Muscle	Evidence of atrophy?	Apoptosis measures	ROS measures	respiration
Hindlimb suspension	Cannavino et al. (2015)	3, 7 and 14 days	C57BL/6J 6-month-old male mice	Gastrocnemius	Lower gastrocnemius cross-sectional area by 3 days of hindlimb suspension.	NA	No change in H_2O_2 emission measured in the absence of substrates and ADP	Lower complex I- (NADH via glutamate and malate) and complex II- ($FADH_2$ via succinate) supported and ADP stimulated respiration by 3 days of hindlimb suspension
	Trevino et al. (2019)	3, 7 and 14 days	C57BL/6J 8-week-old mice (sex unknown)	Soleus	Lower soleus wet weights by 7 days of hindlimb suspension.	Lower complex I and II (NADH via glutamate and malate and $FADH_2$ via succinate) supported and ADP stimulated CRC by 3 days of hindlimb suspension	Higher H_2O_2 emission by 7 days of hindlimb suspension (protocol not disclosed)	Lower complex I- (NADH via glutamate and malate) and complex II- ($FADH_2$ via succinate) supported respiration in the presence of ADP by 3 days of hindlimb suspension
	Yajid et al. (1998)	28 days	Wistar male rats (age unknown)	Gastrocnemius	Lower muscle wet weights (gastrocnemius, soleus, EDL, TA) by 28 days of hindlimb suspension	NA	NA	Lower complex I-supported (NADH via pyruvate and malate) respiration in the presence of ADP in IMF mitochondria of mixed hindlimb tissues (soleus, EDL, TA, gastrocnemius) Lower respiration was also observed from the gastrocnemius muscle alone under the same conditions
Denervation	Adhihetty et al. (2007)	5, 7, 14, 21 and 42 days	Sprague–Dawley male rats (age unknown)	TA	Lower TA, EDL, and gastrocnemius wet weight by 21 days of denervation. TA wet weight further declined by 42 days	Higher rate of complex II-supported ($FADH_2$ via succinate) mPTP opening and lower time to mPTP opening by 14 days post denervation in IMF mitochondria	Higher complex I- (NADH via glutamate) supported H_2O_2 emission in the absence of ADP in TA SS mitochondria by 7 days of denervation. No change in IMF H_2O_2 emission	Lower complex I- (NADH via glutamate) supported and ADP stimulated respiration in SS mitochondria by 7 days of denervation

(*Continued*)

Table 2. (Continued)

Model	Reference	Disuse duration	Species, sex and age	Muscle	Evidence of atrophy?	Apoptosis measures	ROS measures	respiration
Limb immobilization	Edwards et al. (2020)	7 days	Healthy (23 ± 1 years) men	Vastus lateralis	Decreased leg fat-free mass and lower cross-sectional area of type II fibres following 7 days of immobilization	NA	NA	No change in complex I- and II- (NADH via pyruvate, malate and glutamate and $FADH_2$ via succinate) supported respiration in the presence of ADP following 7 days of immobilization
	Gram et al. (2015)	14 days	Healthy young (23 ± 1 years) and older (68 ± 1 years) men	Vastus lateralis	No change in lean body mass	NA	Higher complex I- (NADH via pyruvate and malate) and complex II- ($FADH_2$ via succinate, rotenone) supported H_2O_2 emission in the presence of ADP in both cohorts of men following immobilization	Lower complex I- (NADH via pyruvate and malate) supported respiration in both cohorts of men following immobilization
	Hafen et al. (2019)	10 days	Healthy 18–39 years old men and women	Vastus lateralis	Lower vastus lateralis cross-sectional area	NA	NA	Lower complex I- (NADH via pyruvate and malate) and complex II- ($FADH_2$ via succinate) and ADP stimulated respiration following limb immobilization
	Miotto et al. (2019)	14 days (biopsies taken at 3 and 14 days of immobilization)	Healthy (23 ± 3 years) women	Vastus lateralis	NA	NA	No change in complex II- ($FADH_2$ via succinate) supported H_2O_2 emission in both the presence and absence of ADP at 3 and 14 days following immobilization	Lower complex I- and II- (NADH via pyruvate, malate and glutamate and $FADH_2$ via succinate) supported respiration in the presence of ADP by both 3 and 14 days of immobilization. Decreased submaximal ADP respiration by both 3 and 14 days. No change in palmitoyl-CoA supported respiration in both the presence and absence of malonyl-CoA
	Pileggi et al. (2018)	14 days	Healthy (49.7 ± 3.84 years) men	Vastus lateralis	NA	NA	Higher complex I- (NADH via pyruvate and malate) and complex II-supported ($FADH_2$ via succinate) H_2O_2 emission in the absence of ADP following immobilization	No change in complex I- (NADH via pyruvate and malate) and complex II- ($FADH_2$ via succinate) supported respiration in the presence of ADP following immobilization

(*Continued*)

Table 2. (Continued)

Model	Reference	Disuse duration	Species, sex and age	Muscle	Evidence of atrophy?	Apoptosis measures	ROS measures	respiration
Bed rest	Dirks et al. (2020)	7 days	Healthy (25 ± 1 years) men	Vastus lateralis	Lower body weight	NA	Higher complex II ($FADH_2$ via succinate) and complex I and II (NADH via pyruvate and malate in addition to succinate) maximal H_2O_2 production in the absence of ADP. No changes in ADP stimulated H_2O_2 production. These effects remained when normalized to CS activity. No difference in submaximal H_2O_2 emission	Lower complex I- (NADH via pyruvate, malate and glutamate) and complex II- ($FADH_2$ via succinate) supported and ADP stimulated respiration following bed rest. This effect was lost following normalization to CS activity. Decreased submaximal respiration following bed rest
	Eggelbusch et al. (2024)	60 days (biopsies taken at 6 and 55 days of bed rest)	Healthy (33 ± 9 years) men and women	Vastus lateralis	Progressive loss of whole-body (30 and 60 days), trunk and leg (15 and 60 days) fat-free mass	NA	Lower complex I- (NADH via pyruvate and malate) and complex II- ($FADH_2$ via succinate) supported respiration in the presence of ADP at 55 days of bed rest	NA
	Kenny et al. (2017)	21 days	Healthy (33.7 ± 2.4 years) men	Vastus lateralis	Lower body mass and fat free mass	NA	NA	Lower complex I- (NADH via pyruvate, glutamate and malate) and complex II- ($FADH_2$ via succinate) supported, and ADP stimulated respiration following bed rest. This effect was lost following normalization to CS activity

(*Continued*)

Table 2. (Continued)

Model	Reference	Disuse duration	Species, sex and age	Muscle	Evidence of atrophy?	Apoptosis measures	ROS measures	respiration
	Larsen et al. (2018)	4 days	Healthy (24 ± 4 years) men	Vastus lateralis	No change in lean body mass	NA	No change in complex II- ($FADH_2$ via succinate) supported H_2O_2 production in the absence and presence of ADP normalized to both muscle wet weights and mitochondrial content (citrate synthase activity)	No change in complex I- (NADH via pyruvate, malate, glutamate and malate) and complex II- ($FADH_2$ via succinate) supported and ADP stimulated respiration when normalized to wet weights. However, mitochondrial respiration using the same substrates increased when normalized to mitochondrial content (CS activity used as a marker of mitochondrial content). No change in fatty acid supported (malate and octanoyl-carnitine, ADP) respiration
	Salvadego et al. (2016)	10 days	Healthy (24.1 ± 1.7 years) men	Vastus lateralis	Lower lean body mass	NA	NA	No change in complex I- (NADH via glutamate and malate) and complex II- ($FADH_2$ via succinate) supported and ADP stimulated respiration following 10 days of bed rest
	Salvadego et al. (2018)	21 days	Healthy (27.5 ± 1.7 years) men	Vastus lateralis	Lower body mass, lean body mass and lean thigh mass	NA	NA	Lower complex I- (NADH via glutamate and malate) and complex II- ($FADH_2$ via succinate) supported and ADP stimulated respiration

(*Continued*)

Table 2. (Continued)

Model	Reference	Disuse duration	Species, sex and age	Muscle	Evidence of atrophy?	Apoptosis measures	ROS measures	respiration
	Zuccarelli et al. (2021)	10 days	Healthy (23 ± 5 years) men	Vastus lateralis	Lower body mass; however, no change in lean mass	NA	NA	No change in complex I- (NADH via malate and glutamate) and complex II- ($FADH_2$ via succinate) supported and ADP stimulated respiration following bed rest. Increased respiration at submaximal ADP concentrations following bed rest
Head-down tilt bed rest	Dulac et al. (2024)	14 days	Healthy (60 ± 5 years) men and women	Vastus lateralis	Lower quadriceps volume	No change in complex I- (NADH via glutamate and malate) supported CRC. Decreased mPTP time to opening at 8 days with bed rest; however, this was recovered by 14 days	No change in complex I- (NADH via glutamate and malate) and complex II- ($FADH_2$ via succinate) supported and ADP stimulated H_2O_2 emission	Lower complex I- (NADH via glutamate and malate) and complex II- ($FADH_2$ via succinate) supported and ADP stimulated respiration. No change in fatty acid (malate and palmitoyl-carnitine) supported and ADP stimulated respiration

'NA' indicates the specific mitochondrial function was not measured ('not available'). Disuse durations separated by commas represent separate study groups with varying lengths of disuse. Normalization methods for CRC, ROS and respiration differ between studies. Cases where different normalization techniques influenced reported data are included.
Abbreviations: CRC, calcium retention capacity; EDL, extensor digitorum longus; $FADH_2$, flavin adenine dinucleotide; H_2O_2, hydrogen peroxide; IMF, intermyofibrillar; mPTP, mitochondrial permeability transition pore; NADH, nicotinamide adenine dinucleotide; SS, subsarcolemmal; TA, tibialis anterior.

synthase. During contraction, ATP hydrolysis is increased by numerous ATP-utilizing proteins throughout the muscle fibre, which results in a lower ([ADP][Pi])/[ATP] and greater stimulation of ATP synthase. In this way, mitochondrial respiration is lower when muscle is relaxed and higher when muscles contract. The stimulation of ATP synthesis during contraction helps limit the decline of the ([ADP][Pi])/[ATP] equilibrium. These considerations are critical when interpreting changes in mitochondrial respiration in a given investigation and whether such changes are truly a 'mitochondrial dysfunction'. This theory also highlights the opportunity for future studies to directly measure ([ADP][Pi])/[ATP] to determine whether energy homeostasis is actually compromised and relate this measure to mitochondrial respiration.

As suggested throughout the review, considering substrate-specific responses is an opportunity for interpreting how mitochondria respond to periods of disuse. For example, if disuse lowered skeletal muscle respiration, but respiration was assessed with the use of pyruvate, then the conclusion would be that mitochondrial pyruvate oxidation is reduced. This could be a result of changes in PDH activity, complex I oxidation of NADH, electron flux to complex IV, or changes in the control of oxidative phosphorylation by ADP and Pi, which itself is regulated by specific mitochondrial membrane transporters, and binding to ATP synthase. If a change in pyruvate-supported respiration were related to a parallel change in the content of PDH or its phosphorylation, then new questions arise regarding the link between the intervention and PDH turnover or its post-translational regulation. Likewise, comparisons of carbohydrate *vs.* fatty acid-derived substrates revealed that immobilization does not affect both substrate oxidation pathways the same. Rather, substrate-specific responses were observed where pyruvate-supported respiration was not altered despite increases in fatty acid-supported respiration when normalized to a marker of mitochondrial content (Larsen et al., 2018). This notion of substrate-specific changes in response to disuse was also observed following bed rest, albeit with different substrate comparisons. Specifically, bed rest did not alter fatty acid-supported respiration despite changing glutamate-supported respiration (Dulac et al., 2024). Comparing respiration in response to multiple substrates also provides an opportunity to consider interpretations beyond a respiratory 'dysfunction' where a re-calibration of supply to reduced demand occurred or these 'stress responses' lead to an alternative fate of substrates for reasons yet to be explored (Fig. 3). Finally, comparing mitochondrial respiratory responses to changes in

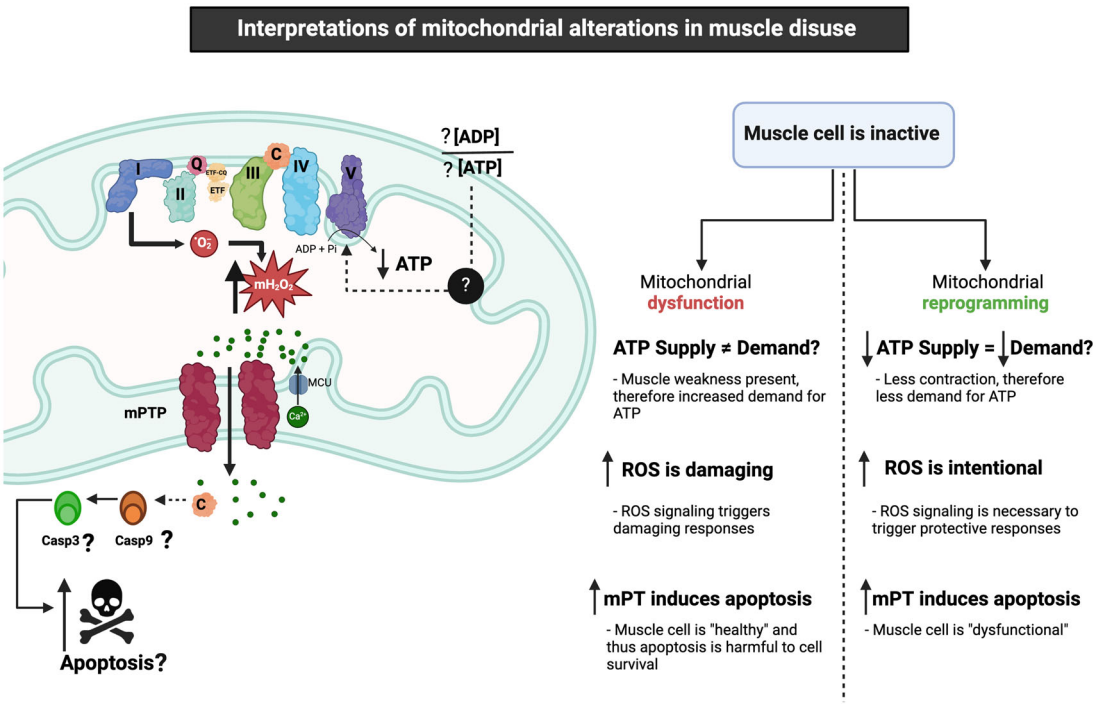

Figure 3. Mitochondrial alterations in non-disease induced muscle disuse
Schematic summary of mitochondrial alterations in non-disease induced muscle disuse: 'dysfunction' or mitochondrial 'adaptation?' C, cytochrome c; Ca^{2+}, calcium; Casp3, Caspase 3; Casp9, caspase 9; ETF, electron transfer flavoprotein; I–V complexes I–V; mH_2O_2, mitochondrial H_2O_2; mPT, mitochondrial permeability transition; mPTP, mitochondrial permeability transition pore; $O_2^{\cdot-}$, superoxide; Pi inorganic phosphate; Q, Q cycle.

mitochondrial content markers provides insight into whether there are 'dysfunctions' within mitochondria or whether mitochondrial quality are maintained amidst changes in volume. For example, people with COPD show normal indices of mitochondrial ATP synthesis when normalized to mitochondrial content (Latimer et al., 2022).

Most of the literature has assessed maximal ADP-stimulated respiration using saturating concentrations of specific substrates. These rates of respiration exceed the low rates of oxidative phosphorylation that occur *in vivo* in resting, inactive muscle. The degree to which decreases in maximal respiration seen throughout the disuse literature would be observed with submaximal concentrations of substrates is unclear. As discussed above, two studies examined mitochondrial respiratory sensitivity to ADP (submaximal respiration) during 7–10 days of bed rest but reported no change or an increase (Dirks et al., 2020; Zuccarelli et al., 2021), whereas one study saw a decrease in respiration by 3 days of immobilization (Miotto et al., 2019). Titrating submaximal concentrations of NADH- and $FADH_2$-generating substrates in the presence of ADP would also allow similar insights into whether the regulation of specific substrate catabolic pathways is altered at kinetics that approach the lower rates seen *in vivo*. Likewise, comparisons of mitochondrial responses to tissue-level assessments of glucose uptake and oxygen delivery could give insight into whether mitochondria are adapting to altered delivery or providing a feedback stimulus to their uptake. For example, resting muscle oxygen uptake is reduced after 10 days of bed rest with decreased mitochondrial respiratory sensitivity to ADP (Zuccarelli et al., 2021), which may reflect a matching of reduced energy demands during disuse coupled to recalibration of mitochondria to a lower rate of oxidative phosphorylation.

Likewise, no investigation has determined whether the increased mitochondrial ROS seen in certain models of disuse trigger cell survival programs that span a variety of cellular functions (Sies & Jones, 2020) (Fig. 3). However, *in vitro* evidence obtained in inactive cultured/living single muscle fibres from mice demonstrates reduced atrophy with agents that target mitochondrial ROS (Skinner et al., 2021). There is considerable opportunity to examine the time-dependent relationship of mitochondrial ROS and cell survival programs (Sies & Jones, 2020) *vs*. catabolic activities through the use of mitochondrial enhancing compounds that are now commercially available (Pin et al., 2022; Szeto & Birk, 2014; Vays et al., 2014).

There is also considerable opportunity to compare mitochondrial responses to measures of muscle strength independent of atrophy during disuse. Many protein targets regulating muscle contraction are known to be ATP-dependent or ROS-sensitive (Bellissimo & Perry, 2020) and could be assessed in relation to the development of weakness over time during disuse. The loss of strength during immobilization is known to be greater than the loss of muscle size (Preobrazenski, Janssen, et al., 2023), although the precise mitochondrial-linked mechanisms regulating loss of strength distinct from atrophy remain unclear.

Disuse is a common characteristic of a variety of diseases that also demonstrate altered mitochondrial functions, including mechanical respiratory ventilation (Kavazis et al., 2009), cortical injury (Hill et al., 2017), cancer cachexia (Brown et al., 2017; Delfinis et al., 2022; Pin et al., 2022; White et al., 2011), neuromuscular diseases (Bellissimo et al., 2022; Mikhail et al., 2023), type 2 diabetes (Mogensen et al., 2007) and many other conditions. Teasing apart the influence of disuse from disease-specific stressors on skeletal muscle mitochondria and their relationship to atrophy could aid in understanding the precise mechanisms of muscle loss. In this regard, the disuse atrophy literature, conducted in healthy and uninjured controls, can provide considerable insight into the effect of inactivity alone and could inspire the inclusion of carefully designed control groups that match reduced physical activity levels of a given population for future directions in each disease-specific research discipline.

Conclusions

Maintaining healthy skeletal muscle is vital for maintaining functional independence. Loss of muscle mass leads to a reduced quality of life and can even lead to death in the context of disease. Muscle inactivity/disuse accelerates muscle loss, but the precise mechanisms remain incompletely understood. Several studies have identified mitochondrial bioenergetic alterations in diverse models of non-disease-induced disuse. These studies have laid the foundation for exploring precise mechanisms by which mitochondrial stress responses contribute to atrophy, or the manner in which such responses may be adaptive in nature. Considering how mitochondria are normally regulated by contraction may lead to new therapeutic paradigms that enhance potential mitochondrial adaptive reprogramming responses, if they occur, or prevent specific forms of mitochondrial 'dysfunction'.

References

Abadi, A., Glover, E. I., Isfort, R. J., Raha, S., Safdar, A., Yasuda, N., Kaczor, J. J., Melov, S., Hubbard, A., Qu, X., Phillips, S. M., & Tarnopolsky, M. (2009). Limb immobilization induces a coordinate down-regulation of mitochondrial and other metabolic pathways in men and women. *PLoS ONE*, **4**(8), e6518.

Adhihetty, P. J., O'Leary, M. F. N., Chabi, B., Wicks, K. L., & Hood, D. A. (2007). Effect of denervation on mitochondrially mediated apoptosis in skeletal muscle. *Journal of Applied Physiology*, **102**(3), 1143–1151.

Baumgartner, R. N., Koehler, K. M., Gallagher, D., Romero, L., Heymsfield, S. B., Ross, R. R., Garry, P. J., & Lindeman, R. D. (1998). Epidemiology of sarcopenia among the elderly in New Mexico. *American Journal of Epidemiology*, **147**(8), 755–763.

Bellissimo, C., & Perry, C. (2020). Regulation of skeletal muscle reactive oxygen species during exercise. In *The Routledge Handbook on Biochemistry of Exercise* (1st ed., pp. 1–20). Taylor & Francis Group.

Bellissimo, C. A., Garibotti, M. C., & Perry, C. G. R. (2022). Mitochondrial stress responses in Duchenne muscular dystrophy: metabolic dysfunction or adaptive reprogramming? *American Journal of Physiology-Cell Physiology*, **323**(3), C718–C730.

Bernardi, P., Carraro, M., & Lippe, G. (2022). The mitochondrial permeability transition: Recent progress and open questions. *Federation of European Biochemical Societies Journal*, **289**(22), 7051–7074.

Bonaldo, P., & Sandri, M. (2013). Cellular and molecular mechanisms of muscle atrophy. *Disease Models & Mechanisms*, **6**(1), 25–39.

Brand, M. D. (2010). The sites and topology of mitochondrial superoxide production. *Experimental Gerontology*, **45**(7-8), 466–472.

Brown, J. L., Rosa-Caldwell, M. E., Lee, D. E., Blackwell, T. A., Brown, L. A., Perry, R. A., Haynie, W. S., Hardee, J. P., Carson, J. A., Wiggs, M. P., Washington, T. A., & Greene, N. P. (2017). Mitochondrial degeneration precedes the development of muscle atrophy in progression of cancer cachexia in tumour-bearing mice. *Journal of Cachexia, Sarcopenia and Muscle*, **8**(6), 926–938.

Cannavino, J., Brocca, L., Sandri, M., Grassi, B., Bottinelli, R., & Pellegrino, M. A. (2015). The role of alterations in mitochondrial dynamics and PGC-1α over-expression in fast muscle atrophy following hindlimb unloading. *The Journal of Physiology*, **593**(8), 1981–1995.

Delfinis, L. J., Bellissimo, C. A., Gandhi, S., DiBenedetto, S. N., Garibotti, M. C., Thuhan, A. K., Tsitkanou, S., Rosa-Caldwell, M. E., Rahman, F. A., Cheng, A. J., Wiggs, M. P., Schlattner, U., Quadrilatero, J., Greene, N. P., & Perry, C. G. R. (2022). Muscle weakness precedes atrophy during cancer cachexia and is linked to muscle-specific mitochondrial stress. *Journal of Clinical Investigation Insight*, **7**(24), e155147.

Delfinis, L. J., Ogilvie, L. M., Khajehzadehshoushtar, S., Gandhi, S., Garibotti, M. C., Thuhan, A. K., Matuszewska, K., Pereira, M., Jones, R. G., Cheng, A. J., Hawke, T. J., Greene, N. P., Murach, K. A., Simpson, J. A., Petrik, J., & Perry, C. G. R. (2024). Muscle weakness and mitochondrial stress occur before severe metastasis in a novel mouse model of ovarian cancer cachexia. *Molecular metabolism*, **86**, 101976.

Dirks, M. L., Miotto, P. M., Goossens, G. H., Senden, J. M., Petrick, H. L., van Kranenburg, J., van Loon, L. J. C., & Holloway, G. P. (2020). Short-term bed rest-induced insulin resistance cannot be explained by increased mitochondrial H_2O_2 emission. *The Journal of Physiology*, **598**(1), 123–137.

Dirks, M. L., Smeets, J. S. J., Holwerda, A. M., Kouw, I. W. K., Marzuca-Nassr, G. N., Gijsen, A. P., Holloway, G. P., Verdijk, L. B., & van Loon, L. J. C. (2019). Dietary feeding pattern does not modulate the loss of muscle mass or the decline in metabolic health during short-term bed rest. *American Journal of Physiology-Endocrinology and Metabolism*, **316**(3), E536–E545.

Du, J., Wang, X., Miereles, C., Bailey, J. L., Debigare, R., Zheng, B., Price, S. R., & Mitch, W. E. (2004). Activation of caspase-3 is an initial step triggering accelerated muscle proteolysis in catabolic conditions. *Journal of Clinical Investigation*, **113**(1), 115–123.

Dulac, M., Hajj-Boutros, G., Sonjak, V., Faust, A., Hussain, S. N. A., Chevalier, S., Dionne, I. J., Morais, J. A., & Gouspillou, G. (2024). A multimodal exercise countermeasure prevents the negative impact of head-down tilt bed rest on muscle volume and mitochondrial health in older adults. *The Journal of Physiology*, Portico. https://doi.org/10.1113/jp285897

Edwards, S. J., Smeuninx, B., Mckendry, J., Nishimura, Y., Luo, D., Marshall, R. N., Perkins, M., Ramsay, J., Joanisse, S., Philp, A., & Breen, L. (2020). High-dose leucine supplementation does not prevent muscle atrophy or strength loss over 7 days of immobilization in healthy young males. *American Journal of Clinical Nutrition*, **112**(5), 1368–1381.

Eggelbusch, M., Charlton, B. T., Bosutti, A., Ganse, B., Giakoumaki, I., Grootemaat, A. E., Hendrickse, P. W., Jaspers, Y., Kemp, S., Kerkhoff, T. J., Noort, W., van Weeghel, M., van der Wel, N. N., Wesseling, J. R., Frings-Meuthen, P., Rittweger, J., Mulder, E. R., Jaspers, R. T., Degens, H., & Wüst, R. C. I. (2024). The impact of bed rest on human skeletal muscle metabolism. *Cell Reports Medicine*, **5**(1), 101372.

Eisenberg, H. A., & Hood, D. A. (1994). Blood flow, mitochondria, and performance in skeletal muscle after denervation and reinnervation. *Journal of Applied Physiology*, **76**(2), 859–866.

Ellis, R. E., Yuan, J., & Horvitz, H. R. (1991). Mechanisms and functions of cell death. *Annual Review of Cell Biology*, **7**(1), 663–698.

Goodpaster, B. H., Park, S. W., Harris, T. B., Kritchevsky, S. B., Nevitt, M., Schwartz, A. V., Simonsick, E. M., Tylavsky, F. A., Visser, M., Newman, A. B., & Study for the HABC. (2006). The loss of skeletal muscle strength, mass, and quality in older adults: The health, aging and body composition study. *The Journals of Gerontology: Series A*, **61**(10), 1059–1064.

Gram, M., Vigelsø, A., Yokota, T., Helge, J. W., Dela, F., & Hey-Mogensen, M. (2015). Skeletal muscle mitochondrial H_2 O_2 emission increases with immobilization and decreases after aerobic training in young and older men. *The Journal of Physiology*, **593**(17), 4011–4027.

Hafen, P. S., Abbott, K., Bowden, J., Lopiano, R., Hancock, C. R., & Hyldahl, R. D. (2019). Daily heat treatment maintains mitochondrial function and attenuates atrophy in human skeletal muscle subjected to immobilization. *Journal of Applied Physiology* , **127**(1), 47–57.

Halliwell, B. (2006). Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiology*, **141**(2), 312–322.

Hargreaves, M., & Spriet, L. L. (2020). Skeletal muscle energy metabolism during exercise. *Nature Metabolism*, **2**(9), 817–828.

Hill, R. L., Singh, I. N., Wang, J. A., & Hall, E. D. (2017). Time courses of post-injury mitochondrial oxidative damage and respiratory dysfunction and neuronal cytoskeletal degradation in a rat model of focal traumatic brain injury. *Neurochemistry International*, **111**, 45–56.

Holwerda, A. M., Dirks, M. L., Barbeau, P. A., Goessens, J., Gijsen, A., van Loon, L. J. C., & Holloway, G. P. (2024). Mitochondrial bioenergetics are not associated with myofibrillar protein synthesis rates. *Journal of Cachexia, Sarcopenia and Muscle*, **15**(5), 1811–1822.

Hyatt, H., Deminice, R., Yoshihara, T., & Powers, S. K. (2019). Mitochondrial dysfunction induces muscle atrophy during prolonged inactivity: A review of the causes and effects. *Archives of Biochemistry and Biophysics*, **662**, 49–60.

Janssen, I., Heymsfield, S. B., & Ross, R. (2002). Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *Journal of the American Geriatrics Society*, **50**(5), 889–896.

Junker, A., Wang, J., Gouspillou, G., Ehinger, J. K., Elmér, E., Sjövall, F., Fisher-Wellman, K. H., Neufer, P. D., Molina, A. J. A., Ferrucci, L., & Picard, M. (2022). Human studies of mitochondrial biology demonstrate an overall lack of binary sex differences: A multivariate meta-analysis. *Federation of American Societies for Experimental Biology Journal*, **36**(2), e22146.

Kavazis, A. N., Talbert, E. E., Smuder, A. J., Hudson, M. B., Nelson, W. B., & Powers, S. K. (2009). Mechanical ventilation induces diaphragmatic mitochondrial dysfunction and increased oxidant production. *Free Radical Biology & Medicine*, **46**(6), 842–850.

Kenny, H. C., Rudwill, F., Breen, L., Salanova, M., Blottner, D., Heise, T., Heer, M., Blanc, S., & O'Gorman, D. J. (2017). Bed rest and resistive vibration exercise unveil novel links between skeletal muscle mitochondrial function and insulin resistance. *Diabetologia*, **60**(8), 1491–1501.

Larsen, S., Lundby, A.-K. M., Dandanell, S., Oberholzer, L., Keiser, S., Andersen, A. B., Haider, T., & Lundby, C. (2018). Four days of bed rest increases intrinsic mitochondrial respiratory capacity in young healthy males. *Physiological Reports*, **6**(18), e13793.

Latimer, L. E., Constantin-Teodosiu, D., Popat, B., Constantin, D., Houchen-Wolloff, L., Bolton, C. E., Steiner, M. C., & Greenhaff, P. L. (2022). Whole-body and muscle responses to aerobic exercise training and withdrawal in ageing and COPD. *European Respiratory Journal*, **59**(5), 2101507.

Leeuwenburgh, C., Gurley, C. M., Strotman, B. A., & Dupont-Versteegden, E. E. (2005). Age-related differences in apoptosis with disuse atrophy in soleus muscle. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, **288**(5), R1288–R1296.

Mcglory, C., Gorissen, S. H. M., Kamal, M., Bahniwal, R., Hector, A. J., Baker, S. K., Chabowski, A., & Phillips, S. M. (2019). Omega-3 fatty acid supplementation attenuates skeletal muscle disuse atrophy during two weeks of unilateral leg immobilization in healthy young women. *The Federation of American Societies for Experimental Biology Journal*, **33**(3), 4586–4597.

Mikhail, A. I., Ng, S. Y., Mattina, S. R., & Ljubicic, V. (2023). AMPK is mitochondrial medicine for neuromuscular disorders. *Trends in Molecular Medicine*, **29**(7), 512–529.

Miotto, P. M., Mcglory, C., Bahniwal, R., Kamal, M., Phillips, S. M., & Holloway, G. P. (2019). Supplementation with dietary ω-3 mitigates immobilization-induced reductions in skeletal muscle mitochondrial respiration in young women. *The Federation of American Societies for Experimental Biology Journal*, **33**(7), 8232–8240.

Mitchell, P. (1961). Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature*, **191**(4784), 144–148.

Mogensen, M., Sahlin, K., Fernström, M., Glintborg, D., Vind, B. F., Beck-Nielsen, H., & Højlund, K. (2007). Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. *Diabetes*, **56**(6), 1592–1599.

Morey-Holton, E. R., & Globus, R. K. (2002). Hindlimb unloading rodent model: Technical aspects. *Journal of Applied Physiology*, **92**(4), 1367–1377.

Mráček, T., Drahota, Z., & Houštěk, J. (2013). The function and the role of the mitochondrial glycerol-3-phosphate dehydrogenase in mammalian tissues. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, **1827**(3), 401–410.

Muller, F. L., Song, W., Jang, Y. C., Liu, Y., Sabia, M., Richardson, A., & Van Remmen, H. (2007). Denervation-induced skeletal muscle atrophy is associated with increased mitochondrial ROS production. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, **293**(3), R1159–R1168.

Nicholls, D. G., & Ferguson, S. J. (2013). *Bioenergetics*, 4th edn. Academic Press, London. https://doi.org/10.1016/C2010-0-64902-9.

Nikoletopoulou, V., Markaki, M., Palikaras, K., & Tavernarakis, N. (2013). Crosstalk between apoptosis, necrosis and autophagy. *Biochimica et biophysica acta*, **1833**(12), 3448–3459.

Oliveira, J. R. S., Mohamed, J. S., Myers, M. J., Brooks, M. J., & Alway, S. E. (2019). Effects of hindlimb suspension and reloading on gastrocnemius and soleus muscle mass and function in geriatric mice. *Experimental Gerontology*, **115**, 19–31.

Perry, C. G. R., & Hawley, J. A. (2018). Molecular basis of exercise-induced skeletal muscle mitochondrial biogenesis: Historical advances, current knowledge, and future challenges. *Cold Spring Harbor perspectives in medicine*, **8**(9), a029686.

Pesta, D., & Gnaiger, E. (2012). High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. *Methods in Molecular Biology*, **810**, 25–28.

Picard, M., Jung, B., Liang, F., Azuelos, I., Hussain, S., Goldberg, P., Godin, R., Danialou, G., Chaturvedi, R., Rygiel, K., Matecki, S., Jaber, S., Des Rosiers, C., Karpati, G., Ferri, L., Burelle, Y., Turnbull, D. M., Taivassalo, T., & Petrof, B. J. (2012). Mitochondrial dysfunction and lipid accumulation in the human diaphragm during mechanical ventilation. *American Journal of Respiratory and Critical Care Medicine*, **186**(11), 1140–1149.

Pileggi, C. A., Hedges, C. P., D'Souza, R. F., Durainayagam, B. R., Markworth, J. F., Hickey, A. J. R., Mitchell, C. J., & Cameron-Smith, D. (2018). Exercise recovery increases skeletal muscle H_2O_2 emission and mitochondrial respiratory capacity following two-weeks of limb immobilization. *Free Radical Biology & Medicine*, **124**, 241–248.

Pin, F., Huot, J. R., & Bonetto, A. (2022). The mitochondria-targeting agent MitoQ improves muscle atrophy, weakness and oxidative metabolism in C26 tumor-bearing mice. *Frontiers in Cell and Developmental Biology*, **10**, 1–13.

Plant, P. J., Bain, J. R., Correa, J. E., Woo, M., & Batt, J. (2009). Absence of caspase-3 protects against denervation-induced skeletal muscle atrophy. *Journal of Applied Physiology*, **107**(1), 224–234.

Preobrazenski, N., Janssen, I., & McGlory, C. (2023). The effects of single-leg disuse on skeletal muscle strength and size in the nonimmobilized leg of uninjured adults: a meta-analysis. *Journal of Applied Physiology*, **134**(6), 1359–1363.

Preobrazenski, N., Seigel, J., Halliday, S., Janssen, I., & McGlory, C. (2023). Single-leg disuse decreases skeletal muscle strength, size, and power in uninjured adults: A systematic review and meta-analysis. *Journal of Cachexia, Sarcopenia and Muscle*, **14**(2), 684–696.

Rasola, A., & Bernardi, P. (2011). Mitochondrial permeability transition in Ca^{2+}-dependent apoptosis and necrosis. *Cell Calcium*, **50**(3), 222–233.

Rosa-Caldwell, M. E., Brown, J. L., Perry, R. A. J., Shimkus, K. L., Shirazi-Fard, Y., Brown, L. A., Hogan, H. A., Fluckey, J. D., Washington, T. A., Wiggs, M. P., & Greene, N. P. (2020). Regulation of mitochondrial quality following repeated bouts of hindlimb unloading. *Applied Physiology, Nutrition and Metabolism*, **45**(3), 264–274.

Salvadego, D., Keramidas, M. E., Brocca, L., Domenis, R., Mavelli, I., Rittweger, J., Eiken, O., Mekjavic, I. B., & Grassi, B. (2016). Separate and combined effects of a 10-d exposure to hypoxia and inactivity on oxidative function in vivo and mitochondrial respiration ex vivo in humans. *Journal of Applied Physiology*, **121**(1), 154–163.

Salvadego, D., Keramidas, M. E., Kölegård, R., Brocca, L., Lazzer, S., Mavelli, I., Rittweger, J., Eiken, O., Mekjavic, I. B., & Grassi, B. (2018). PlanHab(*) : Hypoxia does not worsen the impairment of skeletal muscle oxidative function induced by bed rest alone. *The Journal of Physiology*, **596**(15), 3341–3355.

Seifert, E. L., Estey, C., Xuan, J. Y., & Harper, M.-E. (2009). Electron transport chain-dependent and -independent mechanisms of mitochondrial H_2O_2 emission during long-chain fatty acid oxidation. *Journal of Biological Chemistry*, **285**(8), 5748–5758.

Sies, H., & Jones, D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nature Reviews Molecular Cell Biology*, **21**(7), 363–383.

Siu, P. M., Pistilli, E. E., & Alway, S. E. (2005). Apoptotic responses to hindlimb suspension in gastrocnemius muscles from young adult and aged rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, **289**(4), R1015–R1026.

Skinner, S. K., Solania, A., Wolan, D. W., Cohen, M. S., Ryan, T. E., & Hepple, R. T. (2021). Mitochondrial permeability transition causes mitochondrial reactive oxygen species- and caspase 3-dependent atrophy of single adult mouse skeletal muscle fibers. *Cells*, **10**(10), 2586.

Smeuninx, B., Elhassan, Y. S., Sapey, E., Rushton, A. B., Morgan, P. T., Korzepa, M., Belfield, A. E., Philp, A., Brook, M. S., Gharahdaghi, N., Wilkinson, D., Smith, K., Atherton, P. J., & Breen, L. (2025). A single bout of prior resistance exercise attenuates muscle atrophy and declines in myofibrillar protein synthesis during bed-rest in older men. *The Journal of Physiology*, **603**(1), 87–105.

Stine, Z. E., Schug, Z. T., Salvino, J. M., & Dang, C. V. (2022). Targeting cancer metabolism in the era of precision oncology. *Nature Reviews Drug Discovery*, **21**(2), 141–162.

Szeto, H. H., & Birk, A. V. (2014). Serendipity and the discovery of novel compounds that restore mitochondrial plasticity. *Clinical Pharmacology & Therapeutics*, **96**(6), 672–683.

Thibaudeau, T. A., & Smith, D. M. (2019). A practical review of proteasome pharmacology. *Pharmacological Reviews*, **71**(2), 170–197.

Trevino, M. B., Zhang, X., Standley, R. A., Wang, M., Han, X., Reis, F. C. G., Periasamy, M., Yu, G., Kelly, D. P., Goodpaster, B. H., Vega, R. B., & Coen, P. M. (2019). Loss of mitochondrial energetics is associated with poor recovery of muscle function but not mass following disuse atrophy. *American Journal of Physiology-Endocrinology and Metabolism*, **317**(5), E899–E910.

Vays, V. B., Eldarov, C. M., Vangely, I. M., Kolosova, N. G., Bakeeva, L. E., & Skulachev, V. P. (2014). Antioxidant SkQ1 delays sarcopenia-associated damage of mitochondrial ultrastructure. *Aging*, **6**(2), 140–148.

Wagatsuma, A., Kotake, N., Kawachi, T., Shiozuka, M., Yamada, S., & Matsuda, R. (2011). Mitochondrial adaptations in skeletal muscle to hindlimb unloading. *Molecular and Cellular Biochemistry*, **350**(1–2), 1–11.

Wang, X. H., Zhang, L., Mitch, W. E., LeDoux, J. M., Hu, J., & Du, J. (2010). Caspase-3 cleaves specific 19 S proteasome subunits in skeletal muscle stimulating proteasome activity. *Journal of Biological Chemistry*, **285**(28), 21249–21257.

White, J. P., Baltgalvis, K. A., Puppa, M. J., Sato, S., Baynes, J. W., & Carson, J. A. (2011). Muscle oxidative capacity during IL-6-dependent cancer cachexia. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, **300**(2), R201–R211.

Wicks, K. L., & Hood, D. A. (1991). Mitochondrial adaptations in denervated muscle: Relationship to muscle performance. *American Journal of Physiology*, **260**(4), C841–C850.

Wrogemann, K., & Pena, S. D. (1976). Mitochondrial calcium overload: A general mechanism for cell-necrosis in muscle diseases. *The Lancet*, **307**(7961), 672–674.

Yajid, F., Mercier, J. G., Mercier, B. M., Dubouchaud, H., & Préfaut, C. (1998). Effects of 4 wk of hindlimb suspension on skeletal muscle mitochondrial respiration in rats. *Journal of Applied Physiology*, **84**(2), 479–485.

Zuccarelli, L., Baldassarre, G., Magnesa, B., Degano, C., Comelli, M., Gasparini, M., Manferdelli, G., Marzorati, M., Mavelli, I., Pilotto, A., Porcelli, S., Rasica, L., Šimunič, B., Pišot, R., Narici, M., & Grassi, B. (2021). Peripheral impairments of oxidative metabolism after a 10-day bed rest are upstream of mitochondrial respiration. *The Journal of Physiology*, **599**(21), 4813–4829.

Additional information

Competing interests

The authors declare that they have no competing interests.

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Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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