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INVITED REVIEW

Mitochondrial health and muscle plasticity after spinal cord injury

Ashraf S. Gorgey[1,6,7,12] · Oksana Witt[6] · Laura O'Brien[2] · Christopher Cardozo[8,9,10,11] · Qun Chen[3] · Edward J. Lesnefsky[2,3,4,5] · Zachary A. Graham[8,9]

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Abstract

Mitochondria are responsible for aerobic respiration and large-scale ATP production in almost all cells of the body. Their function is decreased in many neurodegenerative and cardiovascular disease states, in metabolic disorders such as type II diabetes and obesity, and as a normal component of aging. Disuse of skeletal muscle from immobilization or unloading triggers alterations of mitochondrial density and activity. Resultant mitochondrial dysfunction after paralysis, which precedes muscle atrophy, may augment subsequent release of reactive oxygen species leading to protein ubiquitination and degradation. Spinal cord injury is a unique form of disuse atrophy as there is a complete or partial disruption in tonic communication between the central nervous system (CNS) and skeletal muscle. Paralysis, unloading and disruption of CNS communication result in a rapid decline in skeletal muscle function and metabolic status with disruption in activity of peroxisome-proliferator-activated receptor-gamma co-activator 1 alpha and calcineurin, key regulators of mitochondrial health and function. External interventions, both acute and chronical with training using body-weight-assisted treadmill training or electrical stimulation have consistently demonstrated adaptations in skeletal muscle mitochondria, and expression of the genes and proteins required for mitochondrial oxidation of fats and carbohydrates to ATP, water, and carbon dioxide. The purpose of this mini-review is to highlight our current understanding as to how paralysis mechanistically triggers downstream regulation in mitochondrial density and activity and to discuss how mitochondrial dysfunction may contribute to skeletal muscle atrophy.

Keywords Spinal cord injury · Mitochondria · Muscle atrophy · Reactive oxygen species · Peroxisome-proliferator-activated receptor-gamma co-activator 1 alpha (PGC-1 α) · Androgens · Neuromuscular electrical stimulation (NMES) · Functional electrical stimulation (FES) · Resistance training

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✉ Ashraf S. Gorgey
ashraf.gorgey@va.gov

1 Spinal Cord Injury and Disorders, Hunter Holmes McGuire VA Medical Center, Richmond, VA, USA

2 Department of Physiology and Biophysics, Pauley Heart Center, Richmond, VA, USA

3 Division of Cardiology, Department of Medicine, Pauley Heart Center, Richmond, VA, USA

4 Medical Services, Hunter Holmes McGuire VA Medical Center, Richmond, VA, USA

5 Department of Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, VA, USA

6 Department of Physical Medicine and Rehabilitation, Virginia Commonwealth University, Richmond, VA, USA

7 Faculty of Physical Therapy, Cairo University, Giza, Egypt

8 James J. Peters VA Medical Center, Bronx, NY, USA

9 Department of Medicine, Icahn School of Medicine, New York, NY, USA

10 Department of Rehabilitation Medicine, Icahn School of Medicine, New York, NY, USA

11 The Bone Program, Icahn School of Medicine, New York, NY, USA

12 Department of Veterans Affairs, Hunter Holmes McGuire Medical Center, Spinal Cord Injury and Disorders Service, 1201 Broad Rock Boulevard, Richmond, VA 23249, USA

Introduction

Mitochondria are critical to maintain normal skeletal muscle vitality and function. They are involved in ATP synthesis, reactive oxygen species (ROS) generation, and intrinsic apoptotic pathways. The mitochondrial electron transport chain (ETC) is critical for energy generation in cells. The ETC is located within the inner mitochondrial membrane and is composed of four multiprotein complexes (I–IV, Fig. 1). Each complex is composed of multiple subunits encoded by nuclear and mitochondrial genes. The catalytic and regulatory subunits within each complex organize a specific reaction sequence of electron transfers within the complex. The ETC also contains the mobile electron carriers including coenzyme Q and cytochrome *c*. The function of the ETC is to capture the chemical energy released when electrons from NADH or $FADH_2$ are passed to oxygen (Lesnefsky et al. 2016, 2017; Ohnishi et al. 2012).

The captured energy is stored as an electrochemical gradient across the inner membrane [mitochondrial inner membrane (IMM) potential] by transferring protons from the matrix into the intermembrane space. When protons re-enter the matrix through complex V (F1-ATPase), the energy stored in the electrochemical gradient is used to phosphorylate ADP to generate ATP. This process is defined as oxidative phosphorylation. Therefore, the rate of oxidative phosphorylation is affected by the rate of NADH or $FADH_2$ generation, the enzyme activities of the ETC, the integrity of the IMM, and the activity of complex V. Thus, an impaired IMM will decrease ATP production in which the proton gradient between the intermembrane space and the matrix is diminished. A detailed description of mitochondrial physiology can be found in many excellent reviews (Lesnefsky et al. 2016, 2017; Ohnishi et al. 2012).

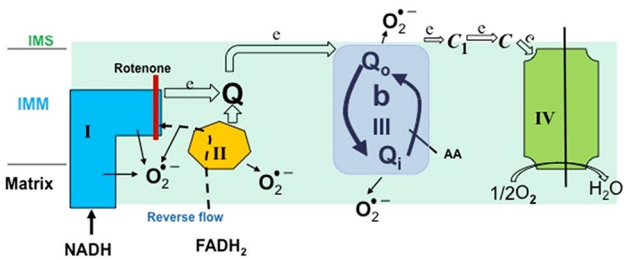

Fig. 1 Schematic depiction of the mitochondrial electron transport chain (ETC). The ETC includes four respiratory complexes (complex I, II, III, and IV). In addition to energy production, the ETC is a major source of ROS generation, particularly Complex I and complex III. Superoxide generated at complex I either through forward electron (e) flow or reverse electron flow is mainly released into the matrix. Inhibition of complex I using rotenone increases the forward electron flow-mediated superoxide generation from complex I, whereas rotenone inhibition decreases the reverse flow-mediated superoxide generation from complex I. Inhibition of complex II can also decrease the reverse flow-mediated superoxide generation from complex I with succinate as the complex II substrate. Inhibition of complex III using antimycin A mainly increases superoxide generation from Qo center. Inhibition of complex IV increases superoxide generation from upstream complexes, especially in complex I. *NADH* nicotinamide adenine dinucleotide, *FADH2* flavin adenine dinucleotide, *IMS* intermembrane space, *IMM* inner mitochondrial membrane, O_2 oxygen; *Q* coenzyme Q, *e* electron flow, *Qo* hydroquinone oxidation site of cytochrome *bc*1, *Qi* quinone reduction site of cytochrome *bc*1, *C1* cytochrome *c*1, *C* cytochrome *c, AA* antimycin A

More recently, other roles for mitochondria have been identified, including initiation of apoptosis, uptake of cytosolic calcium, and generation of ROS which serve both physiologic and pathophysiologic functions. It has also been observed that mitochondria are dynamic organelles, responding to changing cellular energy demands, exercise, diet, and health by fusing to form syncytia known as mitochondrial networks that confer greater efficiency (Laker et al. 2014). These networks may be rapidly broken down through fission to generate individual mitochondrion. Mitochondrial fission and fusion are rapidly regulated by an exquisite and finely tuned set of molecular signals.

The notion that mitochondria become dysfunctional and that they are unable to generate sufficient ATP to meet cellular demands, release excessive amounts of ROS, or both has been gaining attention in recent years. Mitochondrial dysfunction is often seen in cells after damage to the central nervous system by traumatic brain injury (TBI), spinal cord injury (SCI) and neurodegenerative diseases as well as metabolic disorders, such as atherosclerosis, hypertension, insulin resistance, type II DM and obesity, and aging (Chan 2006; Talmadge et al. 2002; Phielix and Mensink 2008). It has been reported that disuse atrophy is associated with reduced numbers of mitochondria, changes in mitochondrial morphology and increased mitochondrial ROS production (Abadi et al. 2009; Adhihetty et al. 2007; Kavazis et al. 2009; Muller et al. 2007; Powers et al. 2011a; Singh and Hood 2011); it was proposed that such mitochondrial dysfunction may result in proteolysis and reduced protein synthesis (Powers et al. 2012; Phillips et al. 2009; Ferrando et al. 1996; Glover et al. 2010; Tesch et al. 2008), suggesting a possible mechanism for skeletal muscle atrophy.

The primary objective of this mini-review is to summarize the current understanding of effects of skeletal muscle paralysis on mitochondria in skeletal muscle. We intend to highlight the effects of SCI on muscle physiology but will also present data from sciatic nerve transections for those cases in which SCI data is lacking (Graham et al. 2018). A sciatic nerve transection is a model of paralysis that, in contrast to spinal cord injury in which the upper motor neuron is injured, results in injury of the lower motor neuron, retraction of the axon from the neuromuscular junction and severe paralysis. While the muscle atrophy resulting from nerve transection resembles that following SCI in many

ways, they are distinct. Atrophy due to denervation is more rapid and associated with more extensive disorganization of the motor end plate and sarcomeric structure. Effects on mitochondrial density and activity will be discussed, as well links to mitochondrial dysfunction, insulin resistance and dyslipidemia. The possibility will be considered that markers of mitochondrial dysfunction appear before appreciable skeletal muscle atrophy and are associated with release by mitochondria of reactive oxygen species (ROS), which leads to protein degradation, proteolysis and ubiquitination.

Loss of mitochondrial number and function after the onset of paralysis occur alongside slow-to-fast twitch fiber-type shifts and muscle atrophy (Wanga and Pessina 2013). This is associated with alterations in levels of proteins known to have combined effects on mitochondrial biogenesis, fiber type and atrophy. Thus, we highlight the mechanisms responsible for skeletal muscle atrophy and slow-to-fast fiber-type switching after SCI including the effects on ubiquitin–proteasome system and how changes in muscle mass and phenotype are altered by androgens. Pathways for degradation of muscle proteins and the upstream signals that activate them will be discussed along with the current gaps in the field. Moreover, the effects of SCI on signaling networks responsible for protein synthesis through Akt, mTORC1, and p70S6 kinase signaling will be discussed as will the current understanding of the mechanisms responsible for the switch from slow-oxidative to fast glycolytic fibers and possible effects of myostatin and de novo expression of connexin 43/45 hemichannels.

Subpopulations of mitochondria

Skeletal muscle has two populations of mitochondria: a subsarcolemmal population (SS) which is located directly underneath the sarcolemma and provides energy for membrane-related events, and an intermyofibrillar population (IMF), which is located within the contractile machinery and is specialized for providing energy for muscle force production and acting as a transient calcium buffer (Cogswell et al. 1993; Romanello and Sandri 2010; Walsh et al. 2006; Rizzuto et al. 2012). Chronic muscle inactivity in humans results in a disorganized mitochondrial reticulum where the IMF population of mitochondria is loosely collected between myofibrils or clustered under the sarcolemma (Kern et al. 2008). In rats that had hindlimb muscles paralyzed by a complete transection of the sciatic nerve, SS mitochondrial depth, a marker of mitochondrial content, was greatly reduced 7 days after paralysis while the IMF area was modestly reduced, suggesting these populations may be regulated differently after paralysis and that this change occurs rapidly (Iqbal et al. 2013). This disorganization likely explains the substantial reductions in metabolic capacity and performance seen in rodent models of SCI (Roy et al. 2002; Kim et al. 2015) and as measured in vivo by 31P magnetic resonance spectroscopy (McCully et al. 2011) and near-infrared spectroscopy (NIRS; Erickson et al. 2013) in humans.

Prolonged skeletal muscle inactivity is associated with SS mitochondria loss (Krieger et al. 1980); remaining SS mitochondria exhibit increased ROS production in atrophying muscles compared to IMF mitochondria (Adhihetty et al. 2007), while IMF mitochondria are more susceptible to apoptotic stimuli (Adhihetty et al. 2005, 2007). Although the exact role and importance of muscle mitochondrial mass in individuals with SCI is not well described, reduced markers of mitochondrial mass and enzyme activity (citrate synthase) were correlated with increased blood lipid levels and decreased ability for glucose handling as well as deterioration in parameters of body composition as measured by magnetic resonance imaging (MRI) and dual energy x-ray absorptiometry (DXA) in persons with chronic SCI (O'Brien et al. 2017a, b). Citrate synthase was positively associated with an increase in thigh muscle cross-sectional area, leg lean mass and was negatively associated with both intramuscular fat and visceral adipose tissue (O'Brien et al. 2017a). These findings suggest that mitochondrial density may play a role in muscle health and metabolic profile after SCI or, conversely, that spasticity or other neuromuscular stimuli that preserve muscle mass are also beneficial to mitochondria and metabolism.

Impaired oxidative capacity

Skeletal muscle atrophy is accompanied by an increase in both absolute and relative intramuscular fat cross-sectional area in those with SCI and other chronic health conditions including glucose intolerance, diabetes mellitus/metabolic syndrome, and cardiovascular disease (Elder et al. 2004; Gorgey and Dudley 2007; Wade and Gorgey 2017). Skeletal muscle oxidative capacity reflects the oxygen uptake and substrate utilization of skeletal muscle tissue, and it is decreased after SCI, aging or reductions in physical activity in humans or rodents (Cartee et al. 2016; Stolle et al. 2018). Uncoupling of mitochondrial respiration due to increased sensitization of the permeability transition pore to apoptosis (Hepple 2016) likely contributes to decreased oxidative capacity in SCI and aged population, but the pathways responsible are still unclear. Given the supporting evidence of the involvement of mitochondria-mediated apoptotic pathways in aging muscle, the possibility of mitochondria-driven myonuclear apoptosis must be considered after SCI although the impact of SCI on the apoptosis-regulating mitochondrial permeability transition pore has yet to be studied. However, it is likely that mitochondrial dysfunction after SCI is accompanied with increased production of ROS

and sensitization of mitochondrial permeability transition pore that leads to increased mitochondrial apoptosis-signaling of protein degradation (Gouspillou et al. 2014). Sensitivity of mitochondrial permeability transition pore was increased in active older adult skeletal muscle despite modest impairment in mitochondrial respiration and production of ROS; these aging-associated changes were associated with threefold increased production in endonuclease G-positive myonuclei as a biomarker of apoptosis (Gouspillou et al. 2014).

Furthermore, paralysis, like other causes of disuse atrophy, leads to a shift in fiber type from a mitochondria-dense, slow and fatigue-resistance oxidative fiber to a fast, fatigable glycolytic fiber which lacks an extensive mitochondrial reticulum (Bodine 2013; Talmadge et al. 2002). In denervation-induced muscle atrophy, the sarcolemma of fast, but not slow, myofibers are permeabilized to small molecules such as Evans blue, ethidium bromide and, most importantly, calcium ions via the de novo expression of connexin (Cx) hemichannels, leading to activation of proteolysis and the inflammasome (Cea et al. 2013). The same study reported that skeletal muscle restricted knockdown of Cx43/Cx45 hemichannels and led to reduced muscle atrophy and reduced NFκB activation, the latter reflective of lowered inflammasome activation (Cea et al. 2013). Thus, Cx hemichannels are potential therapeutic targets to prevent or reduce disuse muscle atrophy.

Previous evidence suggested that mitochondrial dysfunction may precede skeletal muscle atrophy. There is a decrease in mitochondrial oxidative phosphorylation capacity as noted by failure of PCr recovery kinetics following 1 week of SCI in the rat model (Shah et al. 2014). The findings suggested acute mitochondrial dysfunction that may precede or occur during the process of muscle atrophy. Another study showed that functional mitochondrial degeneration precedes the development of muscle atrophy in progression of cancer cachexia in tumour-bearing mice (Brown et al. 2017). The dysfunction in mitochondrial oxidative phosphorylation may result in decreasing mitochondrial fusion and increasing mitochondrial fission, this may ultimately lead to increased mitochondrial autophagy and process of skeletal muscle atrophy. In less than 4 weeks, MitoTimer gene emits green fluorescence in healthy mitochondria shifted to red in the damaged mitochondria. This was accompanied by reduction of mitochondrial respiration and production of H_2O_2 in the permeabilized plantaris muscle (Brown et al. 2017).

Mitochondrial ROS

Several lines of evidence indicate that oxidative stress is involved in muscle atrophy, and that severity of atrophy is dependent on the balance between ROS generation and antioxidant potential. ROS are oxygen-based radicals that are highly reactive and are able to damage proteins, lipids and nucleotides within skeletal muscle by covalent modification (Powers et al. 2011b). Poorly functioning mitochondria have been noted as a primary source of elevated ROS production in aging and diseased skeletal muscles (Powers et al. 2011a). The exact mechanisms behind the increased production of mitochondrial ROS have not been clearly elucidated but excess mitochondrial calcium uptake (Andersson et al. 2011), elevated inflammatory markers (Reid and Moylan 2011), and reduced peroxisome-proliferator-activated receptor-gamma co-activator 1 alpha (PGC1α) expression (St-Pierre et al. 2006) have been proposed as potential factors.

In addition to mitochondria, ROS generation may also be increased in the sarcoplasmic reticulum. Muscle from SCI animals has elevated protein and mRNA levels of NADPH oxidase-4 (NOX4) associated with elevation in amounts of oxidized and nitrosylated ryanodine receptor 1 (RyR1) protein (Liu et al. 2017). Moreover, NOX4-bound RyR1 was observed in muscle from SCI animals suggesting that increased NOX4 expression contributed to RyR1 oxidation and nitrosylation by increasing local ROS generation (Liu et al. 2017). As result of oxidation, nitrosylated RyR1 is unable to bind calstabin 1, a protein critical to RyR1 function. One might assume that these modifications to RyR1 impair function of this calcium channel after SCI. For example, this disruption in calstabin binding creates a leaky sarcoplasmic reticulum (SR) and leads to intracellular calcium imbalance and decreased muscle force generation (Andersson et al. 2011) in mouse models of aging and muscular dystrophies. Thus, ROS from NOX4 may be a key factor to impair muscle contractility in rat muscle after SCI.

The mitochondria are a likely source of a significant portion of ROS generated during muscle atrophy. This is supported by literature in which a mitochondria-targeted antioxidant was able to prevent muscle loss, protects against slow-to-fast fiber conversion and reduces ROS generation in immobilized skeletal muscle (Min et al. 2011) following ventilator-induced diaphragm muscle dysfunction in rats (Powers et al. 2011a). Prolonged muscle inactivity can lead to increased mitochondrial ROS production via excess mitochondrial calcium uptake (Ingalls et al. 1999; Tischler et al. 1990; Weiss et al. 2010; Brookes et al. 2004; Kowaltowski et al. 2009), increase in key proteases (e.g., calpain and caspase-3; Min et al. 2011), increase in fatty acid hydroperoxides (Bhattacharya et al. 2011), impaired protein import into mitochondria (Singh and Hood 2011), and increase in mitochondrial fission (Yu et al. 2011, 2006, 2008; Powers et al. 2012). Live cell imaging has also been used to describe changes in ROS activity during paralysis. Using a transgenic mouse line expressing a mitochondria-targeting yellow fluorescent protein (YFP) that can demonstrate alterations in ROS-related mitochondrial function, spontaneous bursts of

mitochondrial ROS release were seen within 24 h post-denervation in isolated flexor digitorum brevis (FDB) muscle (Karam et al. 2017). A time course of ROS production in mitochondria isolated from denervated rat muscle suggests the SS population of mitochondria produces more ROS than the IMF population compared to controls and that these elevations are seen between 7 and 21 days post-paralysis (Adhihetty et al. 2007). This conclusion is supported in a mouse model of denervation in which elevations in hydrogen peroxide production in isolated mitochondria were observed beginning at 5 and 7 days post-paralysis, associated with reductions in the respiratory control ratio, a ratio between state 3 respiration (when substrate is added) and state 4 respiration (when all ADP has been converted to ATP) (Muller et al. 2007; Pollock et al. 2017). Interestingly, decreased markers of ROS have been seen in denervated mice on a calorically restricted diet and diet restriction-related atrophy was limited by overexpression of glutathione peroxidase 4 (Bhattacharya et al. 2009). Denervated muscle fibers may also influence surrounding innervated fibers to increase their ROS production by release from denervated fibers of pro-inflammatory cytokines (Pollock et al. 2017).

Mitochondria effect on muscle atrophy

Reactive oxygen species produced by mitochondria can lead to increased proteolysis and decreased protein synthesis that leads to muscle atrophy. Caspase-3 may be activated indirectly by mitochondrial ROS, calpains by increased cytosolic calcium, and polyubiquitination and proteasomal degradation by indiscriminant oxidation of proteins; moreover, E3 ligase expression is increased by oxidative stress (Li et al. 2003; McClung et al. 2008; Powers et al. 2011a, c; Whidden et al. 2010). Oxidative stress also decreases protein synthesis by decreasing activity of Akt/mTOR (Powers et al. 2011c; Fig. 2); Akt has molecular control of the transcription factors forkhead box protein 1 and 3 (FOXO1 and FOXO3). FOXOs upregulate gene expression of muscle-specific RING finger protein 1 (MuRF1) and muscle atrophy F-box protein (MAFbx), two muscle-specific ubiquitin ligases involved in targeting muscle proteins to the proteasome during muscle atrophy (Bodine 2013; Bonaldo and Sandri 2013). Signaling via mechanistic target of rapamycin (mTOR) and ribosomal protein S6 kinase (p70S6K) is decreased in humans with SCI (Bodine 2013; Bonaldo and Sandri 2013). mTORC1 is the master regulator of protein synthesis and, thus, is one key determinant of muscle mass (Hoppeler 2016). It can be activated by branched chain amino acids, insulin and growth factors (Dodd and Tee 2012), and its activation has been shown to correlate with increase in muscle protein synthesis rate (Phillips 2009). Signaling in response to mechanical inputs to muscle, such as those occurring in response to high-load exercises, can activate the mTOR/raptor complex known as mTORC1 via mechano-transducer molecules such as focal adhesion kinase (FAK) (Goldmann 2014; Janostiak et al. 2014) and is required for IGF-1-mediated muscle growth (Crossland et al. 2013). FAK is believed to be a key upstream mediator in mechanotransduction and its levels are temporally and positively correlated with p70S6k (Klossner et al. 2009). Of interest when considering the slow-to-fast fiber-type switch after SCI is the observation that FAK is also linked to slow twitch muscle fiber phenotypes and the expression of oxidative genes and proteins (Durieux et al. 2009). We have noted that total and phosphorylated FAK are reduced at 56 days post-SCI associated with marked reductions in activated p70S6k protein expression after a complete spinal cord transection in male rats (Graham et al. 2016). Others have shown that low levels of neuromuscular electrical stimulation (NMES) in chronically injured individuals with SCI have strong FAK phosphorylation response (Yarar-Fisher et al. 2014), suggesting FAK may be a key regulator in detecting membrane stretch in paralyzed tissue even years after injury.

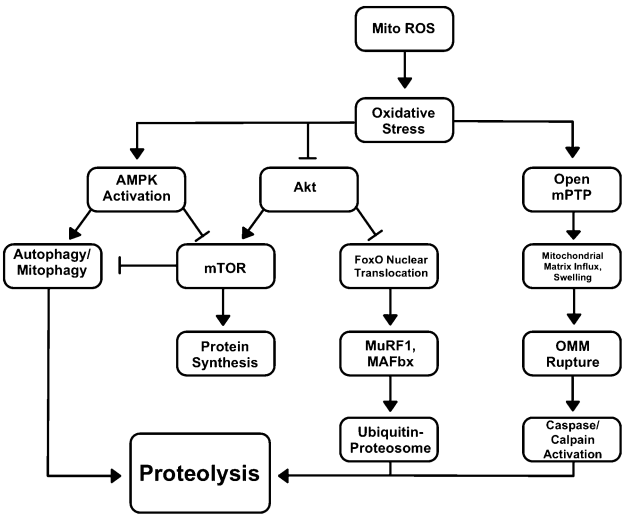

Fig. 2 Summary of the effects of oxidative stress on mitochondrial function. ROS (reactive oxygen species) produced by mitochondria can lead to increased proteolysis and decreased protein synthesis that leads to muscle atrophy. ROS production can also lead to mitochondrial outer membrane permeabilization, that causes dispersal of proapoptotic factors, leading to activation of caspase-3 and muscle protein breakdown with myonuclear apoptosis. *Akt* protein kinase B, *mTOR* mechanistic target of rapamycin, *FOX01* Forkhead box protein 01, *FOX03* Forkhead box protein 03, *ROS* reactive oxygen species, *AMPK* adenosine monophosphate kinase, *MuRF1* muscle-specific RING finger protein 1, *MAFbx* muscle atrophy F-box protein, *mPTP* mitochondrial permeability transition pore, *OMM* outer mitochondrial membrane, *mito* mitochondria

ROS production can also lead to mitochondrial outer membrane (MOM) permeabilization, which causes dispersal of proapoptotic factors (Adhihetty et al. 2005; Kavazis

et al. 2008), leading to activation of caspase-3 and muscle protein breakdown with myonuclear apoptosis and decreased protein synthesis. Animal models without caspase-3 showed protection against muscle atrophy and myonuclei loss (Plant et al. 2009). Opening of the mitochondrial permeability transition pore (mPTP), a non-selective pore in the IMM, is a key step to induce apoptosis (Halestrap and Richardson 2015; Juhaszova et al. 2009). mPTP opening is triggered by oxidative stress and calcium over load, leading to an influx of water and calcium into the mitochondrial matrix causing mitochondrial swelling and mitochondrial rupture, triggering apoptosis. Key proteins in the apoptotic program include cytochrome *c*, apoptosis inducing factor (AIF), and endonuclease G (Marzetti et al. 2010; Fig. 2). mPTP opening is increased in aged muscles undergoing atrophy. In isolated mitochondria from denervated muscles, the sensitivity to mPTP opening is increased due to increased cyclophilin D expression (Marzetti et al. 2008; Halestrap 2009). Interestingly, the sensitivity to mPTP opening is not altered in physically active people with aging (Cartee et al. 2016).

Finally, mitochondrial damage due to muscle inactivity can further lead to decreased ATP production and protein synthesis with increased proteolysis (Kavazis et al. 2009; Max 1972; Min et al. 2011; Powers et al. 2011a; Romanello et al. 2010). The increased proteolysis is associated with AMP-activated protein kinase (AMPK)-activated FOXO3 expression, leading to elevated expression of MAFbx, MURF-1, LC3, and Bnip3 (Greer et al. 2007; Romanello et al. 2010; Fig. 2). AMPK senses cellular ATP and nutrient levels and regulates levels of anabolic versus catabolic processes in response to changes in ATP (Mounier et al. 2015). It inhibits mTORC1 activity to decrease protein synthesis, a very energy-consuming biosynthetic process, but increases glucose uptake and fatty acid oxidation; it promotes an oxidative muscle phenotype by phosphorylating regulatory proteins and transcription factors and co-activators, including PGC-1α (Hoppeler 2016).

Mitochondrial fusion and fission

The mitochondria in skeletal muscle undergo fusion to form a tightly regulated reticulum that increases the efficiency of oxidative phosphorylation (Glancy et al. 2015). Organization of this network through fusion and fission (division of mitochondria into two or more independent structures) events is driven by the state of the muscle fiber and is meant to ensure there is a sufficient capacity for energy production during demanding activities such as exercise while, presumably, limiting excessive oxidative stress resulting from inappropriately high mitochondrial-usage activities (Laker et al. 2014). Increased fission via Dynamin-related protein-1 (Drp 1) and Fission protein 1 (Fis1) is associated with increased apoptosis in certain cell types and increased atrophy in skeletal muscle fibers (Romanello et al. 2010; Youle and Karbowski 2005) (Fig. 3). Mitochondrial fusion is regulated by the outer membrane fusion proteins mitofusion 1 and 2 (Mfn 1–2) and inner membrane fusion protein optic atrophy 1 (Opa1). Reductions in these proteins are associated with mitochondrial dysfunction and muscle atrophy (Chen et al. 2010; Olichon et al. 2003). AMPK and the histone/protein deacetylase Sirtuin (SIRT1) respond to ATP levels and nutrient availability in a cell and regulate cellular metabolism, gene expression, and mitochondrial function (Ruderman et al. 2010) (Fig. 4). In denervation-induced atrophy, inhibition of Drp1 and Fis1 leads to protection against muscle wasting, while mitochondrial fission increases release of proapoptotic factors and mitochondrial ROS production via activation of FoxO3 and NF-kB (Romanello et al. 2010). Expression of activated Drp1 and mitochondrial fission factor 1 (MFF1) are elevated at 21 days post-sciatic nerve transection and remain elevated out to 35 days with no changes in total protein levels, suggesting that paralysis leads to a sustained fission response in effected muscle (Graham et al. 2018).

Autophagy

Autophagy is a process by which cellular organelles and membranes are directed to the lysosome for degradation. In rodents, denervation-induced muscle atrophy is mediated both by elevations in non-lysosomal proteolysis and

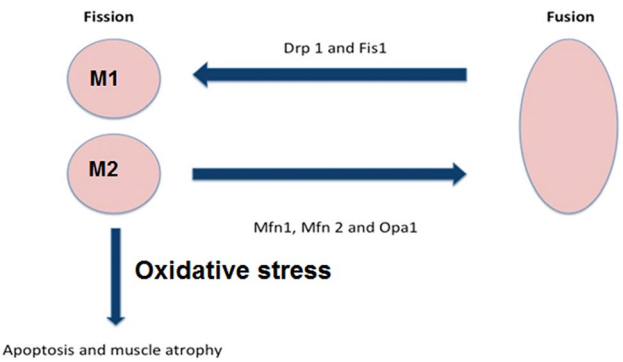

Fig. 3 Mitochondrial function through the process of fission and fusion of mitochondria. Mitochondria in skeletal muscle undergo fusion and fission in response to the state of the muscle fiber to ensure there is a sufficient capacity for energy production during demanding activities. Fission via Drp 1 (Dynamin-related protein-1) and Fis1 (Fission protein 1) is associated with increased apoptosis in certain cell types and increased atrophy in skeletal muscle fibers. Mitochondrial fusion is regulated by the Mfn 1–2 (mitofusion 1 and 2) and Opa1 (optic atrophy 1). Reductions in these proteins are associated with mitochondrial dysfunction and muscle atrophy. *Drp 1* dynamin-related protein-1, *Fis1* fission protein 1, *Mfn 1* mitofusin-1, *Mfn 2* mitofusin-2, *Opa1* optic atrophy-1

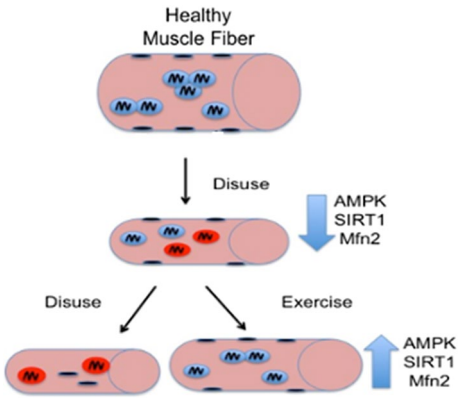

Fig. 4 Muscle fiber mitochondria in response to disuse (red colored mitochondria) and exercise (blue colored mitochondria). Disuse of skeletal muscle from immobilization or unloading mechanistically triggers downstream regulation of mitochondrial density and activity. Disuse atrophy is associated with reduced numbers of mitochondria, changes in mitochondrial morphology and increased mitochondrial ROS production. AMPK (adenosine monophosphate kinase), (Mfn-2) Mitofusin-2, and SIRT1 (Sirtuin 1) respond to nutrient availability in a cell and regulate the metabolism, gene expression, and mitochondrial function. *AMPK* adenosine monophosphate kinase, *SIRT1* Sirtuin 1, *Mfn2* Mitofusin-2

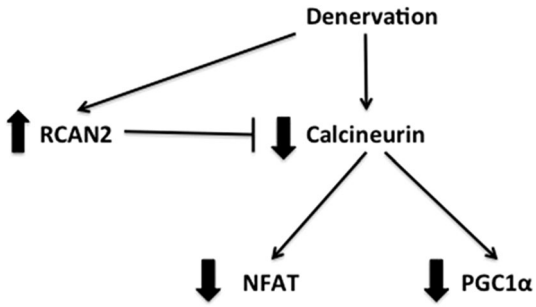

Fig. 5 Effects of denervation on calcineurin and regulator of calcineurin 2. Calcineurin helps maintain an oxidative muscle profile by regulating the downstream activation and resultant nuclear translocation of the nuclear factor of activated T-cell (NFAT) family, with elevated levels of calcineurin and distinct NFAT isoforms being associated with oxidative muscle fibers. Denervation is associated with reduction of calcineurin activity and elevations in protein expression of RCAN2 (regulator of calcineurin 2), a protein that inhibits calcineurin activity. *NFAT* nuclear factor of activated T-cell, *RCAN2* regulator of calcineurin 2, *PGC1α* peroxisome-proliferator-activated receptor-gamma co-activator 1 alpha

autophagy. In rat skeletal muscles, autophagy is increased 10 weeks after a complete spinal cord transection and in mice paralyzed with a sciatic nerve transection, we noted progressive elevation across 35 days in expression of activated Unc-like kinase 1 (Ulk1) and additional downstream makers of autophagy/mitophagy (Graham et al. 2018). In both rodents and humans, paralysis-induced muscle atrophy includes two phases: a rapid loss within 1–2 weeks in rodents and 6 weeks in humans, with slow rates thereafter. Relative contributions of proteolysis via the ubiquitin–proteasome pathway, autophagy and reduced protein synthesis to muscle atrophy after SCI remain to be established. Markers of autophagy are reduced sub-acutely-to-chronically after SCI and may subject to increase early after injury (Martin-Rincon et al. 2018).

Biochemical and metabolic regulators of mitochondria in response to unloading or exercise

Several proteins have been identified as key regulators of mitochondrial health and function. Disuse atrophy results in a rapid change in the transcriptome and resultant proteome which ultimately targets major contractile and scaffolding proteins for proteolytic destruction by cytosolic proteases, primarily through the ubiquitin–proteasome pathway, or by autophagy, in the immediate time-frame after the muscle is immobilized (Bodine 2013). SCI and denervation from a sciatic nerve transection are unique forms of disuse atrophy in which there is substantial or complete disruption in tonic communication with the innervating motor neuron which is likely a compounding source of muscle atrophy in individuals with paralysis. Reduced mitochondrial viability, due to reductions in biogenesis and elevations in mitophagy and production of ROS, could be key mechanisms that lead to the atrophy and dysfunction seen in muscle after paralysis. On the other hand, exercise has been shown to increase mitochondrial mass and activity and improve insulin sensitivity in able-bodied individuals (Papa 1996).

Calcineurin

One of the established hallmarks of disuse atrophy is the oxidative-to-glycolytic shift in muscle fiber type. Calcineurin helps maintain an oxidative muscle profile by regulating the downstream activation and resultant nuclear translocation of the nuclear factor of activated T-cell (NFAT) family, with elevated levels of calcineurin and distinct NFAT isoforms being associated with oxidative muscle fibers (Naya et al. 2000; Calabria et al. 2009; Fig. 5). Expression and activity of calcineurin have been shown to be reduced 2 months after hindlimb paralysis following a sciatic nerve transection in rats compared to sham animals (Qin et al. 2014). Denervation is also associated with elevations in protein expression of regulator of calcineurin 2 (RCAN2), a protein that inhibits calcineurin activity, at two months post-paralysis (Qin et al. 2014; Garton et al. 2014; Fig. 5). However, a complete SCI-reduced mRNA levels of RCAN1 and 2 in female rats and this difference in outcomes is likely due to the difference between the effects of axon retraction after denervation as

compared to preserved neural connections at the neuromuscular junction on skeletal muscle after SCI, which results in different types of post-injury skeletal muscle gene expression (Wu et al. 2013; Zeman et al. 2009).

PGC1α

Calcineurin also regulates the activity of myocyte enhancer factor 2 (MEF2) (Wu et al. 2001), a key transcription factor that drives the expression PGC1α (Handschin et al. 2003). PGC1α is commonly referred to as a master regulator of mitochondrial biogenesis due to its capacity in regulating important mitochondrial genes for mitochondrial fusion (Hood et al. 2016) and its ability to promote formation of slow, oxidative fibers (Lin et al. 2002). In multiple denervation studies, reductions in PGC1α protein and mRNA expression have been noted (Adhihetty et al. 2007; Sandri et al. 2006). SCI also results in reduced slow myosin and PGC1α mRNA expression and reduced PGC1α total and nuclear-localized protein expression (Wu et al. 2012, 2013). Reduced expression of PGC1α in a transgenic mouse model results in poorly formed muscle mitochondria (Vainshtein et al. 2015). Accordingly, over-expressing PGC1α in a muscle-specific manner protects muscle fiber cross-sectional area after denervation and preserves function (Sandri et al. 2006). Thus, maintaining PGC1α expression can be used to protect muscle health and metabolic function.

II. Targeting mitochondria may attenuate muscle atrophy

The targeting of the mitochondria may be a therapeutic approach to attenuate muscle atrophy in persons with SCI or to evoke hypertrophy in rehabilitative settings. This approach may to lead to subsequent improvements in body composition and metabolic profile. Those with SCI are prone to insulin resistance and glucose intolerance (Bauman and Spungen 2001) as well as other co-morbidities that may affect skeletal muscle metabolic function (Bank et al. 2015; Davies et al. 2007; Cirnigliaro et al. 2015). While positive mitochondrial health is generally associated with positive insulin sensitivity and improved glucose handling, there is no conclusive evidence as to whether insulin resistance can be caused by faulty mitochondria or vice versa (Hesselink et al. 2016). Despite the many perturbations described in muscle paralyzed by SCI (e.g., Kern et al. 2004), muscle maintains a remarkable capacity to respond to loading to improve markers of metabolic function. Six months of triweekly body weight-supported treadmill training was able to elevate GLUT4 protein levels, hexokinase activity and muscle glycogen and phosphocreatine (PCr) levels (Phillips et al. 2004). While these are largely glycolytic markers, the elevation in PCr is important as it is a major store of potential energy within skeletal muscle due to the mitochondrial PCr shuttle (Glancy et al. 2015). Furthermore, there are strong associations between mitochondrial density and enzyme activity and parameters of body composition and metabolic profile after SCI (O'Brien et al. 2017a, b).

NMES and FES as modalities to improve mitochondrial parameters

Exercise is a cornerstone in the rehabilitation of persons with SCI (Table 1). Surface neuromuscular electrical stimulation (NMES) and functional electrical stimulation (FES) are commonly used for training paralyzed muscles (Gorgey and Shepherd 2010; Gorgey et al. 2012; Gorgey and Lawrence 2016). NMES training is associated with increased muscle cross-sectional areas (Carvalho de Abreu et al. 2008; de Abreu et al. 2009; Mahoney et al. 2005), improvement in plasma glucose level (Mahoney et al. 2005; Arija-Blázquez et al. 2014) and circulating insulin (Gorgey et al. 2012) following training. FES endurance training is associated with improvements in blood glucose control (Chilibeck et al. 1999; Griffin et al. 2009; Mohr et al. 2001; Jeon et al. 2002; Hjeltnes et al. 1998), increase in lean muscle mass and motor and sensory ability (Giangregorio et al. 2012; Griffin et al. 2009; Scremin et al. 1999), improvement in inflammatory bio-markers (Griffin et al. 2009), and increase in glycolytic and mitochondrial oxidative enzyme activities (Kjaer et al. 2001). Of note, the distribution of changes was related to the proximity of muscles to the electrodes with no correlation between the number of exercise sessions and the extent of muscle hypertrophy (Scremin et al. 1999). Similarly, body weight-supported treadmill training (BWSTT) increases muscle mass and oxidative capacity and decrease total and low-density lipoprotein cholesterol (Giangregorio et al. 2005, 2006; Stewart et al. 2004). Finally, locomotor training (LT) results in increased muscle size with improved voluntary activation (Jayaraman et al. 2008). Due to the heterogeneity of study designs and small sample size, it is hard to define the best exercise prescription. However, exercise interventions may prevent and treat metabolic disorders and improve mitochondrial health after SCI.

Chronic training using twitch pulses of FES for 5 days per week over 4 months in patients at least 9 months post-SCI was able to restore myofibril assembly, T-tubule and sarcoplasmic reticulum ultrastructure, and reduce aberrant mitochondrial clustering (Kern et al. 2004). In a separate study, chronic FES resulted in greater protein levels of PGC1α (Gorgey et al. 2017). Training of paralyzed muscles of those with SCI using FES was associated with changes in the expression of multiple genes responsible for fatty acid metabolism and mitochondrial ribosomes (Lammers et al.

Table 1 Representative studies that conduct exercise with emphasis on muscle, metabolic and mitochondrial health in persons with SCI

Studies	Sample size	Dose	Major findings
Hjeltnes et al. (1998)	5 individuals with complete motor SCI (level C5–C7, ASIA A), all males, aged 28–44, 4–23 years post-injury	Electrically stimulated leg cycling: 7 sessions/week for 8 weeks	Increase in whole-body insulin-stimulated glucose uptake with increase in insulin-stimulated 3-*O*-methylglucose transport Increase in protein expression of GLUT4, glycogen synthase, and hexokinase II Increase in hexokinase II activity Improved muscle fiber type distribution and fiber
Chilibeck et al. (1999)	5 individuals with motor-complete SCI (level C5–T8, ASIA A), 4 men and 1 woman, aged 31–50 years, 3–25 years post-injury	Functional electrical stimulation (FES)-leg cycle ergometer training, 30 min on 3 sessions/week for 8 weeks	Increased GLUT-1 and GLUT-4 Increased muscle oxidative capacity (citrate synthase activity) Improved insulin sensitivity index
Scremin et al. (1999)	13 individuals with neurologically complete motor sensory SCI (level C5–L1, ASIA A), all males, aged 24–46, 2–19 years post-injury	3-phase FES-induced ergometry exercise program: for an average of 52.8 weeks	Increases in muscle CSAs Increase in the ratio of muscle to adipose tissue of lower extremities
Kjaer et al. (2001)	10 individuals with SCI (level C6–T4, ASIA A), 8 men and 2 women, aged 27–45, 2–24 years post-injury	FES of an upright cycling motion: 3 sessions/week for 12 months (intense training), followed by 6 months of training 1 session/week (reduced training)	Increase in glycolytic (hexokinase, lactate dehydrogenase) and mitochondrial oxidative (citrate synthase, 3-hydroxyacyl-CoA dehydrogenase) enzyme activities with intense training and maintained by reduced training
Mohr et al. (2001)	10 individuals with SCI (level C6–T4, ASIA A), 8 men and 2 women, aged 27–45 years, 3–23 years post-injury	FES cycling 3 sessions/week for 1 year (intensive training), with 7 subjects continuing training 1 session/week for next 6 months (reduced training)	Increase in insulin sensitivity and GLUT 4 content in skeletal muscle after intensive training and decreased again with reduced training
Jeon et al. (2002)	7 individuals with motor complete SCI (level C5–T10), 5 males and 2 females, aged 30–53 years, 3–40 years post-injury	Electrical stimulation-assisted cycling: 30 min/day, 3 sessions/week for 8 weeks	Lower glucose level at 2 h OGTT Improvement in glucose utilization and insulin
Stewart et al. (2004)	9 individuals with a traumatic SCI (level C4–T12, ASIA C), 8 men and 1 woman, mean time 8.1 years post-injury	BWSTT: 3 sessions/week for 6 months	Decrease in externally supported body weight Increase in walking velocity and duration of the training sessions Increase in the mean muscle-fiber area of type I and IIa fibers Decrease in type IIax/IIx fibers and IIX myosin heavy chain Increase in type IIa fibers Increase muscle oxidative capacity (citrate synthase and 3-hydroxy-acyl-CoA dehydrogenase) Improved blood lipid profile
Giangregorio et al. (2005)	5 individuals with traumatic SCI (level C3–C8, ASIA B-C), 2 males and 3 females, aged 19–40, 66–170 days post-injury	BWSTT: 2 sessions/week for total of 48 sessions over 8 months	Increase in muscle CSA Reduction in bone mineral densities at almost all lower limb sites No changes in bone geometry or bone biochemical markers

Table 1 (continued)

Studies	Sample size	Dose	Major findings
Mahoney et al. (2005)	5 individuals with chronic, complete SCI (level C5 to T10, ASIA A), all males, mean age 35 years, mean time 13.46 years post-injury	Resistance exercise training with NMES: 2 sessions/week for 12 weeks	Increase in quadriceps femoris CSA Trend for a reduction in plasma glucose levels
Giangregorio et al. (2006)	13 individuals with incomplete SCI, mean age 29 years, 1–24 years post-injury	Body weight-supported treadmill training (BWSTT): 3 sessions/week for 12 months	Increase in whole-body lean mass and muscle CSA at the thigh and lower leg sites Decrease in whole-body bone density No changes in bone density/bone geometry at any site, or in bone biochemical markers
Carvalho de Abreu et al. (2008)	15 individuals with SCI (level C4–C7, ASIA A), all males, mean age 31.95, mean time 66.43 months post-injury	Treadmill gait training with neuromuscular electrical stimulation (NMES): for 6 months, 2 sessions/week for 20 min. Control group performed only conventional physiotherapy	Increase of quadriceps cross-sectional area (CSA) even with a partial body weight support provided
Jayaraman et al. (2008)	5 individuals with chronic incomplete SCI (level C4–T12, ASIA C-D), 4 men and 1 woman, A, aged 21–50, 8–39 months post-injury	Locomotor training: 5 sessions/week for 9 weeks	Improved lower extremity skeletal muscle function and muscle size Improved ability to generate both peak and instantaneous torque by extensor muscles about the ankle and knee joint
de Abreu et al. (2009)	15 individuals (level C4–C7, ASIA A), all males, mean age 32.3, mean time 64.1 months post-injury	NMES: 2 sessions/week for 20 min each time for 6 months by the gait group. Traditional therapy: for 6 months and then gait training without NMES for additional 6 months by the control group	Increase of quadriceps CSA in the intervention group
Griffin et al. (2009)	18 individuals with complete and incomplete SCI, (level C4–T7), aged 25–57, 1–53 years post-injury	FES cycling: 2–3 sessions/week for 10 weeks	Increase in total FES cycling power and work done Increase in lean muscle mass Increase in ASIA motor and sensory scores for the lower extremity Decrease in blood glucose and insulin levels following the OGTT Decrease in levels of IL-6, TNF-alpha, and CRP No change in bone and adipose mass, triglyceride levels
Giangregorio et al. (2012)	34 individuals with chronic incomplete SCI (level C2–T12, AIS C-D), more than 18 months post-injury	FES-assisted walking (intervention), or aerobic and resistance training (control) sessions 3 sessions/week for 16 weeks	No change in body composition (whole body and leg lean mass and whole-body fat mass) Increased muscle area change score
Arija-Blázquez et al. (2014)	8 individuals with recent SCI (ASIA A), all males, 8 weeks post-injury	Electromyostimulation: 47 min/day, 5 sessions/week on both muscle and bone for 14 weeks	Increase in quadriceps muscle CSA Glucose and insulin peaks moved forward after the training No changes in basal levels of bone biomarkers Similar bone losses and levels of lipid and lipoprotein

2012). Other forms of NMES alter the acute gene expression array profiles of additional mitochondrial and oxidative regulators in chronically injured SCI patients (Petrie et al. 2014, 2015). Three hours of FES upregulates expression of genes that drive expression of mitochondrial genes for fat and carbohydrate metabolism but not those for slow, oxidative muscles. Extended periods of FES of 1-year increase expression of genes involved in metabolizing fats and carbohydrates to generate ATP, as well as oxidative slow twitch muscle genes (Petrie et al. 2015).

Previous work revealed that 12 weeks of NMES-evoked resistance training (NMES-RT) using ankle weights increased muscle cross-sectional area by 35% in individuals with SCI (Gorgey et al. 2012). Another study revealed that only 8 weeks of twice-weekly NMES-RT reversed 48 weeks of atrophy and restored knee extensor muscle size to 75% that of able-bodied controls (Dudley et al. 1999). The major pathway by which resistance training induces muscle hypertrophy is by activation of mTOR and Akt. (Fig. 6). An acute bout of NMES-RT has been shown to increase IGF and Akt signaling in skeletal muscle of individuals with SCI (Yarar-Fisher et al. 2014). This suggests that paralyzed muscle remains responsive to mechanical loading long after injury; however, the signaling cascades following NMES-RT have not been fully characterized.

Resistance training also results in increased PGC-1α4, a PGC-1α isoform involved in muscle hypertrophy (Ruas et al. 2012). PGC-1α4 has been shown to increase IGF and decrease myostatin, a potent negative regulator of muscle mass. In addition to muscle hypertrophy, decreased myostatin has been shown to increase production of irisin, a metabolic hormone that increases energy expenditure (Ruas et al. 2012). Therefore, the PGC-1α4 signaling pathway activated by resistance training may induce both muscle hypertrophy and improve whole-body energy expenditure. Twelve weeks of resistance training increased serum irisin almost two-fold in older adults and irisin concentration was negatively related to percent body fat (Zhao et al. 2017). The effect of electrical stimulation on the genes that were regulating muscle mass was previously studied including MyoD, atrogin-1, MuRF1 and Myostatin. The expression of these genes increased following denervation and was decreased following electrical stimulation in denervated rat muscle (Russo et al. 2010). Individuals with chronic SCI have significantly higher serum myostatin and sclerostin which has been suggested to be a biomarker of bone resorption and osteoporosis levels, compared with healthy controls (Invernizzi et al. 2015). Another study has shown significant decrease in protein levels of FoXO1, FoXO3a and atrogin-1 and decrease in mRNA levels of atrogin-1, MuRF1 and myostatin in individuals with chronic complete SCI, indicating internal mechanism for reducing further muscle protein loss in SCI (Léger et al. 2009).

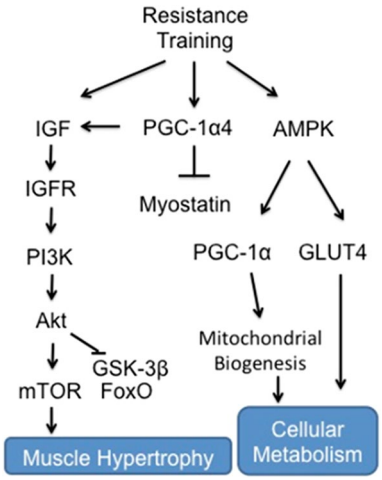

Fig. 6 Skeletal muscle signaling pathways activated in response to resistance training. Resistance training induces muscle hypertrophy by activation of protein synthesis through the mammalian target of rapamycin (mTOR) protein complex via insulin growth factor receptor (IGFR)-induced protein kinase B (Akt) by phosphoinositide 3-kinase (PI3K). In addition to increasing protein synthesis, the IGF-1-Akt-mTOR signaling pathway also inhibits pathways involved in protein degradation, including glycogen synthase kinase-3β (GSK-3β) and forkhead transcription factor (FOXO). *IGF* insulin growth factor, *IGFR* insulin growth factor receptor, *PI3K* phosphoinositide 3-kinase, *mTOR* mechanistic target of rapamycin, *GSK-3β* glycogen synthase kinase-3β, *FOXO* forkhead transcription factor, *PGC1α* peroxisome-proliferator-activated receptor-gamma co-activator 1 alpha, *AMPK* adenosine monophosphate kinase, *GLUT4* glucose transporter type 4

Previous studies have shown that 8 weeks of resistance training resulted in increased skeletal muscle-mitochondrial proteins and mRNA and 12 weeks increased complex I and II-driven mitochondrial respiration in able-bodied individuals (Porter et al. 2015). Similarly, NMES-RT increased muscle oxidative capacity by 25% in individuals with SCI, as measured by phosphocreatine recovery (Ryan et al. 2013). Additionally, NMES increased muscle oxidative capacity three-fold as measured by near-infrared resonance spectroscopy (NIRS), a convenient and relatively inexpensive technique to measure mitochondrial activity at rest and in response to exercise (Erickson et al. 2013, 2017).

Although measuring oxygen consumption of permeabilized muscle fibers by high-resolution respirometry (Fig. 7) is currently the gold standard for assaying skeletal muscle mitochondrial function, muscle biopsies are not feasible for clinical trials with large sample sizes and are associated with discomfort and complications due to their invasive nature. As little as 4 weeks of NMES increased succinate dehydrogenase activity (mitochondrial complex II), while a longer training period also increased the proportion and size of oxidative muscle fibers (Rochester et al. 1995). Even a single session of NMES increased skeletal muscle genes involved in oxidative phosphorylation and mitochondrial remodeling

in individuals with SCI (Petrie et al. 2014). AMPK is an important regulator of cellular energy homeostasis and activates pathways involved in glucose uptake (GLUT4) and mitochondrial biogenesis (PGC-1α) (Coughlan et al. 2014). A previous study from our laboratory suggests that AMPK, PGC-1α, and GLUT4 protein expression are increased in response to 16 weeks of NMES-induced cycling (Gorgey et al. 2017). An increase in skeletal muscle mitochondrial function may translate to increased daily energy expenditure and reduced adiposity in addition to improved glucose utilization and metabolic profile.

Androgens

It has been well established that supraphysiological doses of androgens can improve muscle size and function (Bhasin et al. 1996). In an SCI rodent model, testosterone plus nandrolone attenuated the loss in body and muscle weight 2 months after injury (Wu et al. 2012). This same report showed reduction in activation of Smad2 and Smad3, which are signal transduction molecules acting downstream of the myostatin receptor Activin receptor IIB, and preserved PGC1α and slow troponin mRNA and protein expression of (Wu et al. 2012). In a denervation model, testosterone plus nandrolone resulted in greater gastrocnemius and soleus weights compared to sham animals associated with elevated calcineurin activity and calcineurin A protein expression but reduced RCAN2 protein expression (Qin et al. 2014). Thus, androgens seem to be able control key aspects of the oxidative fiber type post-SCI.

Summary and conclusion

It is well accepted that SCI results in slow-to-fast fiber switching which may be associated with altered mitochondrial function. Given that mitochondrial respiration is responsible for most ATP production, it is logical to propose that altered mitochondrial function may explain the rapid fatigue observed in muscle post-SCI. The discovery that individual mitochondria fuse to form extended networks that generate ATP more efficiently, and that such networks are rapidly disassembled in response to changes in specific cellular cues such as nutrient availability, provides a physiologic mechanism to fine-tune mitochondrial function at the organellar level. Mitochondrial fission machinery is activated after nerve transection suggests that a similar disassembly of the mitochondrial reticulum may occur after SCI and provides a plausible mechanism to explain the fatigue observed in muscle post-SCI despite relatively unchanged levels of key mitochondrial enzymes. Mitochondria are now recognized as having central roles in pathogenesis of atrophy secondary to disuse through increased release of ROS. Interventions that prevent mitochondrial ROS release are potential approaches that may preserve muscle mass and function after SCI. It has been suggested that the use of antioxidants may maintain cellular redox homeostasis by elimination of ROS as well as protect against immobilization-induced

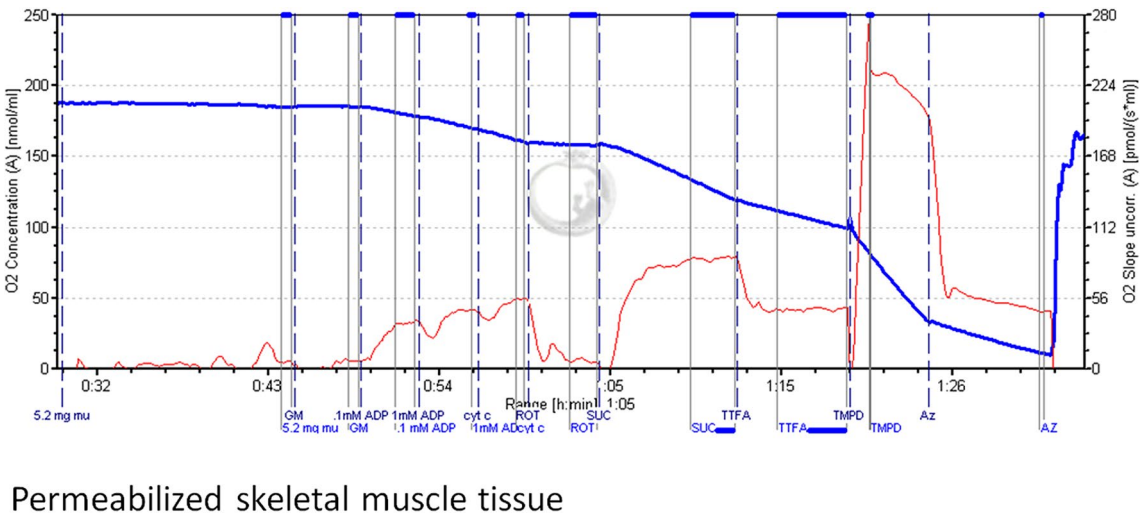

Fig. 7 A representative tracing for oxidative phosphorylation determination. The rate of oxidative phosphorylation in permeabilized skeletal muscle was measured in Oroboros. The blue line is oxygen concentration in oxygen chamber, and the red line is the rate of oxygen consumption. Glutamate + malate (GM), succinate, and *N, N, N′, N′*-tetramethyl-*p*-phenylenediamine + ascorbate (TMPD) were used as complex I, II, and IV substrates, respectively. Rotenone (ROT), Thenoyltrifluoroacetone (TTFA), Azide (AZ) were used as the complex I, II, and IV inhibitor, respectively. ADP was used to stimulate oxygen consumption. The final rate of oxidative phosphorylation was normalized by per-mg skeletal muscle

muscle atrophy (Min et al. 2011; Powers et al. 2011a). It is likely that derangements in mitochondrial mass, structure and function post-SCI are a direct consequence of unloading with resultant declines in levels of key regulatory factors such as PGC-1α and decreased activation of such factors through AMPK or kinase cascades activated by muscle contraction. Conversely, accumulating evidence demonstrates that physical activity achieved by partial body weight-supported treadmill training or NMES at least partially reverses deleterious effects of SCI on markers of mitochondrial mass. Such interventions also appear to upregulate Glut4 expression and improve insulin action in some studies. Future investigations investigating effects of these rehabilitation interventions on formation of mitochondrial reticulum and mitochondrial function should be informative with regard to cellular mechanisms by which benefits of electrical stimulation of paralyzed muscle are achieved. Investigations of the potential of therapies that reduce mitochondrial ROS production in SCI models should also be informative from both a mechanistic and therapeutic point of view.

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