[Frontiers in Bioscience, Landmark, 20, 157-172, January 1, 2015]

The role of mitochondrial fusion and fission in skeletal muscle function and dysfunction

Sobia Iqbal[1], David A. Hood[1]

[1]School of Kinesiology and Health Science, and Muscle Health Research Center, York University, Toronto, Ontario M3J 1P3, Canada

TABLE OF CONTENTS

1. ABSTRACT

Classic textbook depictions of mitochondria portray these organelles to be static bean-shaped structures. However the mitochondrial population is quite heterogeneous, and can form small individual organelles or extended reticula throughout muscle. This morphological plasticity is controlled by fission and opposing fusion events. Skeletal muscle mitochondrial morphology has been demonstrated to be altered under various disease conditions, including diabetes, denervation, as well as during development, aging, and exercise. This implies that mitochondrial fission and fusion machinery components are involved in regulating the architecture of the organelle during various states of muscle use and disuse. Furthermore, disruptions in either of these opposing processes have been demonstrated to result in diseases, suggesting that proper maintenance of mitochondrial morphology is critical for proper cell function.

2. INTRODUCTION

In 1890 Altmann first characterized mitochondria based on their morphological resemblance to bacteria, and termed them “bioblasts” (4). However, it was Carl Benda that described the structures and coined the name mitochondria (mito-tread, chondrion-granule). The morphology of these organelles began to be detailed in 1915 by Lewis and Lewis (63). They noted that “in living cells these bodies are never quiet, but continually changing in shape, size, and position.” Bakeeva *et al* first described the existence of a mitochondrial reticulum in rat diaphragm muscle (9). Subsequently, Kirkwood *et al* demonstrated that mitochondria exist as a reticulum in skeletal muscle and adapt to endurance exercise through enhanced proliferation of the reticulum, concomitantly with an increase in oxidative capacity of the muscle (56; 57). Ogata and Yamasaki noted differences in the three-dimensional mitochondrial

structures in muscle fibres possessing different oxidative capacities (74).

3. THE IMPORTANCE OF MITOCHONDRIAL FISSION AND FUSION

3.1. Existence of mitochondrial reticulum

The distribution, volume, and shape of mitochondria within muscle fibres are a reflection of the balance between the activities of mitochondria morphology proteins, possibly regulated by the requirement for metabolite transport, energy demand, and oxygen diffusion. The beneficial aspects of a continuous membrane network would be apparent in instances where the energy requirement and substrates are not homogeneously distributed within a fiber. A reticular mitochondrial structure could rapidly compensate for any deficits, serving to maintain an electrical potential along the membranes for extended distances, and reducing diffusion times for lipid soluble metabolites and oxygen (9). Fusion also enables complementation to occur as a cellular defense mechanism (77). Healthy, non-mutated mitochondrial genomes are able to complement potential defects that mutated mtDNA may confer through inter-mitochondrial mixing. In addition to the advantages of an extended reticulum, there is the potential disadvantage that regional dysfunction might transfer to the entire organelle network. As such, it is important that mitochondria continuously undergo cycles of fission and fusion. Fission events can mitigate mitochondrial damage by segregating dysfunctional regions, thus minimizing potentially widespread detrimental effects. Furthermore, the complete loss of mitochondrial fusion results in a pathogenic profile in skeletal muscle, whereby mtDNA mutations are severely elevated (19). Romanello *et al* (88) also found that increases in mitochondrial fragmentation contribute to muscle loss, and that the inhibition of organelle fission could protect from muscle atrophy. Disruptions in organelle dynamics have also been tied to obesity and type 2 diabetes (64). As detailed later, these studies suggest that mitochondrial morphology is linked in the pathophysiology of various diseases.

Skeletal muscle possesses two reasonably distinct populations of mitochondria based on their composition and biochemical properties. Subsarcolemmal (SS) mitochondria are located just below the sarcolemma, while intermyofibrillar (IMF) mitochondria are found between the myofibrils. Adopting subcellular fractionation techniques originally developed for heart muscle (78), Krieger *et al* (59) were the first to separate the two mitochondrial subfractions from skeletal muscle. Mitochondria in both regions exhibit subtle differences in their biochemical properties, allowing for differences in respiration, lipid composition, enzyme activities, and protein synthesis rates. The SS mitochondria are situated nearest the capillary, and are thought to contribute to the energy requirements for O_2 transport to the muscle cells. Conversely, IMF mitochondria are thought to provide energy for the contractile apparatus of muscles. Picard *et al* (82) provided a detailed characterization of the SS and IMF mitochondrial morphologies, and noted that several morphological parameters were divergent between the two subpopulations, such as circularity, form factor and perimeter. SS mitochondria were consistently observed to be more circular, whereas the IMF subpopulation adopted more of an elongated shape. Unpublished results from our laboratory have shown that the expression of the fusion proteins is greater in the IMF, compared to the SS subfraction within rat skeletal muscle. Furthermore, we have also found similar rates of fission and fusion events in C2C12 myoblasts (unpublished observations). These results support the concept that mitochondrial morphologies are not randomly distributed, and are likely preserved and regulated.

3.2. Machinery involved in mitochondrial reticulum formation

Changes in mitochondrial dynamics are regulated by opposing fusion and fission processes. Fusion of smaller organelles promotes the intermixing of mitochondrial material and an expansion of the mitochondrial reticulum, an observation made by Kirkwood *et al* some years ago (57) as a result of endurance training. Fission, on the other hand, divides mitochondria into smaller pieces. Essential mammalian skeletal muscle fusion proteins involved in these processes include mitofusin 2 (Mfn2), which behaves as an anchor, bringing adjacent mitochondria into close enough proximity to fuse their outer membranes, as well as the inner mitochondrial membrane protein optical atrophy 1 (Opa1). Mitochondrial membrane fission is mediated by dynamin related peptide 1 (Drp1), in combination with fission protein 1 (Fis1). Fis1 is involved in outer membrane fission, whereas Drp1 must be recruited from the cytosol to the outer surface of the organelle, whereupon it wraps around the constriction points of dividing mitochondria to promote the division of organelles (Figure 1).

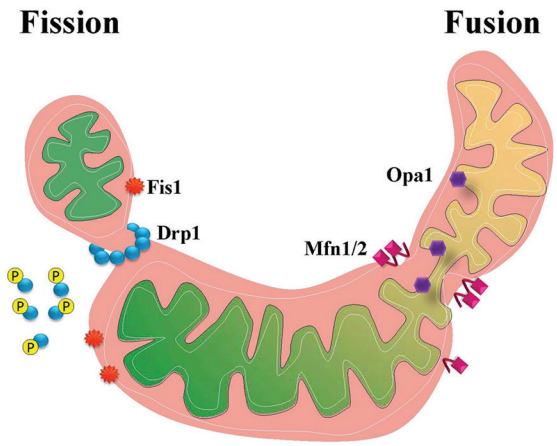

Figure 1. The maintenance of mitochondrial shape is dependent on reaching an equilibrium between the processes of fission and fusion. Fusion involves the mixing of mitochondrial material, whereas fission divides the organelle into smaller components. Mitochondrial fusion is governed by the proteins Opa1 and Mitofusin 1 and 2 (Mfn1/2). Opa1 coordinates the fusion of the inner mitochondrial member (IMM), whereas Mfn1 and 2 tether adjacent organelles and bring them in close proximity to one another to allow for the fusion of the outer mitochondrial membranes (OMM). Fission events are mediated by Fis1 and Drp1. Fis1 resides on the OMM and Drp1 is found in the cytosol in a phosphorylated state. During mitochondrial division, Drp1 is dephosphorylated and recruited to the organelle. Drp1 forms a ring-like structure to constrict and divide the mitochondrion.

3.2.1. Mfn1/2

The double membrane nature of mitochondria makes it essential to consider both components when discussing mitochondrial morphology. Inner and outer membrane fusion events within mitochondria are separable, and involve different morphology machinery proteins. Outer member fusion is regulated by the mitofusin isoforms, Mfn1 and Mfn2 (87). The mitofusins contain a GTP-binding domain, a transmembrane domain, and two coiled-coil regions, upstream and downstream of the transmembrane domain that allow for anchorage of the protein in the outer membrane (90). The mitofusins have N- and C-terminal domains exposed towards the cytosol. This topology implies that the hydrophobic domains span the outer membrane twice (87). Mitofusins tether adjacent mitochondria through a dimeric anti-parallel coiled-coiled structure, either through homotypic (Mfn2-Mfn2) or heterotypic (Mfn1-Mfn2) means (58), thus allowing for adjacent organelles to be brought into close proximity to one another for a fusion event to occur. Although structurally similar, the functional roles of the mitofusins are divergent. Mfn2 is presumed to have more of a regulatory role, while Mfn1 is mainly responsible for tethering adjacent mitochondria (50). Moreover, Mfn1 can only promote mitochondrial fusion in the presence of Opa1, whereas Mfn2 can act independently (21). The expression patterns of the mitofusins are tissue-specific, whereby Mfn2 is highly expressed in skeletal muscle as compared to Mfn1 (89).

3.2.2. Opa1

Inner mitochondrial membrane fusion is mediated by the dynamin-related protein, Opa1. Opa1 is weakly anchored to the inner mitochondrial membrane, with the N-terminus exposed to the matrix and the bulk of the C-terminal domain exposed to the intermembrane space (28; 76). Mammalian Opa1 exists as eight isoforms as a result of alternative splicing and proteolytic processing (25), which may indicate functional diversity of the protein, however the differences in function and processing mechanisms have yet to be fully identified. Separately, the Opa1 isoforms have little activity, but when co-expressed the long and short and isoforms functionally complement each other (28). The yeast homologue, mitochondrial genome maintenance protein 1 (Mgm1), exists in two lengths, the large Mgm1 (l-Mgm1) and the small isoform (s-Mgm1). Both forms are required for the proper maintenance of mitochondrial DNA and morphology (42). Thus, it seems reasonable to postulate that manipulating the ratios of the Opa1 isoforms may affect mitochondrial morphology in mammals.

Over-expression of Opa1 promotes an elongated mitochondrial reticulum, whereas knockdown of the protein results in fragmented organelles (21). Blockage of the protein in mammalian cells eliminates all mitochondrial fusion, and results in poor cell growth and decreased respiration (17). In addition to the role of Opa1 in inner mitochondrial membrane fusion, Opa1 is also essential for mitochondrial cristae organization and function. In cells devoid of this protein, their mitochondria exhibit disorganized cristae structures (75).

3.2.3. Drp1

Similar to mitochondrial fusion, fission is also regulated by specific morphology proteins. Mitochondrial fission was first observed as a process orchestrated by dynamin related protein 1 (Drp1) (12; 96). Drp1 belongs to a family of large GTPase of the dynamin family, a group of highly conserved proteins known for their ability to self-assemble and oligomerize. Drp1 polymerizes into

a ring-like structure around mitochondria. Cross-bridging of the GTPase domains of adjacent Drp1 proteins results in GTP hydrolysis, constriction, and ultimately the severing of mitochondria (98). Drp1 can be modified post-translationally through phosphorylation, sumoylation and/or ubiquitylation. The phosphorylation status of Drp1 is an important determinant of its localization and effect on mitochondrial structure. Phosphorylation of Drp1 at Ser^{637} results in inhibition of its GTPase activity and an inability of Drp1 to translocate from the cytosol to the sites of mitochondrial division, resulting in an attenuation of mitochondrial fission (16; 22). Because Drp1 lacks a mitochondrial targeting sequence, it is thought that other proteins may be responsible for mediating the recruitment of Drp1 to mitochondria. Whether or not the other well characterized fission protein Fis1 is involved, is still controversial.

Cyclic AMP-dependent protein kinase (PKA)-dependent phosphorylation of Drp1 at Ser^{637} results in inhibition of its GTPase activity and mitochondrial division (16; 22). Additionally, in response to ATP depletion, de-phosphorylation of Drp1 at Ser^{637} is upregulated, but becomes re-phosphorylated when cells are returned to a high ATP environment, indicating that Drp1 phosphorylation is ATP-dependent (20). ATP depletion results in Drp1 activation and contributes to mitochondrial fragmentation (20). Moreover, it has been shown that calcineurin regulates Drp1 dephosphorylation, allowing for its translocation to mitochondria (14; 22). Knockdown of calcineurin results in an accumulation of P-Drp1 in the cytosol, coinciding with reduced Drp1 localization to mitochondria and attenuated mitochondrial fragmentation (105).

Drp1 is expressed at the highest levels in the brain, followed by skeletal and cardiac muscles (96). Depletion of Drp1 in HeLa cells leads to the loss of mtDNA, a decrease in mitochondrial respiration, coupled to an increase in the levels of cellular reactive oxygen species (79). Moreover, inhibition of Drp1 prevents DNA fragmentation, indicative of reduced apoptotic cell death (31). Therefore, these results support the importance of Drp1 and mitochondrial fission.

3.2.4. Fis1

The other well characterize fission protein is mitochondrial fission protein 1 (Fis1). The C-terminal domain of Fis1 contains a single transmembrane domain that anchors the protein in the mitochondrial outer membrane, leaving the bulk of the protein exposed to the cytosol. This N-terminal cytosolic domain contains six α-helices and is responsible for the fission activity of Fis1 (27; 94; 100). The transmembrane segment of Fis1 directs the import of the protein post-translationally from the cytosol into the outer membrane of mitochondria (54). Over-expression of Fis1 results in fragmented mitochondria, whereas the knockdown of the protein leads to an increase in organelle networks and interconnected mitochondria (97). Interestingly, inhibition of Fis1 significantly reduces cell death, to a greater extent than the down-regulation of Drp1 (62). Drp1 and Fis1 are both involved in mitochondrial fission, although their involvement in non-morphology roles in the cell may be through divergent pathways.

Taken together these findings propose that multiple members of the mitochondria fusion-fission machinery are involved in controlling mitochondrial morphology, and that there is a complex interplay between the regulators. It is likely that the predominant morphology of mitochondria within the cell is determined by the ratio of fission-to-fusion proteins, as well as intracellular signaling events which determine their location and activity. The identification of signaling pathways, as well as other components of the fission and fusion machinery, will help us further understand the complexities which define organelle morphology and function within cells.

3.3. Models of mitochondrial reticulum alterations in skeletal muscle

Mitochondria are vital contributors not only to cellular life, but also to the death process. Disruptions in mitochondrial dynamics can lead to developmental defects and diseases (108), suggesting that proper maintenance of mitochondrial morphology is critical for normal cell function. As outlined below, skeletal muscle mitochondrial morphology has been demonstrated to be altered under various disease conditions, such as diabetes (6; 53), and denervation (68), as well as during development (8), aging (47), and exercise (57; 81).

3.3.1. Alterations in mitochondria morphology with development and age

Immediately following birth, a progressive increase in the formation of interconnected mitochondrial networks occurs (8). During this phase of muscle development, mitochondria begin to take on the appearance of mature reticula. They begin to

form mitochondrial junctions with branched tubular networks (8). It is speculated that mitochondrial fusion during this process is important for life, since mice deficient in Mfn2 experience severe placental disruptions and homozygous Mfn2 deficiencies are embryonic lethal (18).

Mitochondrial dynamics are not only important during early development, but also during the aging process. Age affects the size of mitochondria in skeletal muscle, with mitochondrial area being larger in infancy, and gradually becoming smaller with age in humans (52). Poggi *et al* (84) found similar results in their human study, with mitochondrial size and volume per fibre area decreasing with age. Our laboratory has also found that the IMF mitochondria are more fragmented with age, and that the layer of SS mitochondria is markedly reduced in senescent Fisher 344 Brown Norway rats as compared to their younger counterparts (47). This alteration in mitochondrial morphology was likely due to the altered ratio of fission to fusion proteins in the muscle of older animals. We observed an upregulation in the expression of Fis1 and Drp1, and a reduction in Mfn2, resulting in fragmented mitochondria in the aged animals (49).

Aging is a multidimensional process that involves a decline in the ability of the muscle to adapt to stress, damage and disease. A decline in muscle mass and strength are prominent features of aged skeletal muscle, a condition known as sarcopenia. Skeletal muscle mass is determined by the rate of muscle protein synthesis and breakdown. A reduction in muscle mass may occur if degradation rates exceed synthesis, or conversely if a decrease in muscle protein synthesis occurs.

Several mechanisms have been proposed that cause sarcopenia, including increased reactive oxygen species (ROS) and oxidative stress (15; 104), an accumulation of mitochondrial abnormalities such as mtDNA mutations (65; 104), as well as an increased susceptibility to apoptosis (15; 26). The free radical theory of aging postulates that the accumulation of ROS is a key determinant of an organism's life span (40). Although the mechanisms by which ROS contributes to aging and reduced lifespan are still unclear, there is evidence that increased oxidative stress damages DNA, proteins, and lipids (11). Oxidative stress can damage mtDNA and impair mitochondrial function, resulting in an accumulation of ROS and exacerbated intracellular ROS-induced damage. Mitochondria isolated from senescent skeletal muscle display increased ROS production, in addition to enhanced apoptotic susceptibility (15; 66). Fan *et al* (29) demonstrated that oxidative stress induces mitochondrial fragmentation in myoblasts. We observed that the mitochondrial fragmentation resulting from oxidative stress was mediated, in part, by Drp1. We found that Drp1 became dephosphorylated and subsequently participated in mitochondrial fragmentation in the presence of elevated oxidative stress (48). These findings indicate that oxidative stress is a vital signaling mechanism in the regulation of mitochondrial morphology and may contribute to age-related mitochondrial fragmentation.

3.3.2. Alterations in mitochondria morphology with denervation

Similar to the effects of aging, chronic muscle disuse brought about by denervation is a potent stimulus that reduces mitochondrial content in the muscle (2; 68; 95). The molecular basis mediating this alteration has been attributed to, at least in part, the decreased expression of nuclear genes encoding mitochondrial proteins (NUGEMPs) (95), reductions in protein import (95), increased ROS production (73), and/or increased mitochondrially mediated cell death (2).

Denervation occurs when the nerve supply to muscle is severed. It can be achieved through mechanical means (i.e. surgical sectioning or crushing), chemical blocking (i.e. tetrodotoxin, TTX), or injury (70). This condition results in the elimination of nerve-muscle communication. A predominant feature of denervation is muscle atrophy and a loss of muscle strength (106). Moreover, denervation can lead to changes in the ultrastructure of muscle, including alterations to the number and size of mitochondria, sarcomere disorganization, and myofibrillar disruptions (67). Mitochondrial content, as well as protein levels within tissue are balanced between the rate of synthesis and degradation. With denervation there is a decrease in mitochondrial biogenesis and enhanced mitochondrially-mediated apoptosis (106). With denervation mitochondrial import machinery components and import rates are reduced (95), resulting in coordinated decreases in the expression of both mitochondrially-encoded subunit III and nuclear-encoded subunit VIc components of cytochrome c oxidase (COX) (106).

The exact mechanisms governing the loss in muscle mass with denervation have yet to be fully elucidated, although there is increasing

evidence suggesting an upregulation in the rate of muscle protein degradation and the activation of mitochondrial-specific autophagy (termed mitophagy). For this process to occur it is thought that mitochondria must first be processed into smaller, fragmented components in order to be engulfed by autophagosomes during the mitophagy process. In diaphragm muscle following denervation, it has been observed that mitochondria are smaller in structure, in contrast to control muscle that contain long, reticular organelles (68). Recently we observed that denervation increased the ratio of fission to fusion proteins in rat skeletal muscle (49). Aging, another form of muscle atrophy, also displays an upregulation in fission protein expression, albeit through a divergent pathway (49). Mitochondrial fragmentation has been observed to occur with muscle atrophy, in particular following denervation.

3.3.3. Alterations in mitochondria morphology with mitochondrial biogenesis

Skeletal muscle has the remarkable ability to adapt to increased metabolic demands, such as exercise, by increasing the size and number or mitochondria. This process is termed mitochondrial biogenesis and it involves the coordination of both mitochondrial and nuclear genomes (44). Changes in mitochondrial biogenesis are due to multiple molecular events. These pathways include the activation of signaling kinases, the induction of transcription factors, changes in mRNA stability, post-translational modifications, alterations in protein import rates, and/or the rate of protein folding (44).

In response to exercise there is an upregulation in the mRNA and protein expression of the transcriptional coactivator PGC-1α and transcription factors, such as mitochondrial transcription factor A (Tfam) and nuclear response factors (NRF-1,2) (3; 35; 60). Activation of transcriptional factors regulates the expression of nuclear genes encoding mitochondrial proteins (NUGEMPs), as well as the transcription of PGC-1α (45). In turn, PGC-1α can bind to transcription factors, thereby also regulating the expression of NUGEMPs (83). The coordination of both the nuclear and mitochondrial genomes allows for the expansion of the mitochondrial reticulum and alterations in the composition of the mitochondrion, thus allowing for adequate adaptations during the rising energy demands brought about by exercise (43, 57).

Acute exercise does not appear to have any effect on the extensiveness of the mitochondrial reticulum. The size and morphology of mitochondria remains unaltered despite an increase in muscle mitochondrial membrane interactions (81). Thus, an acute bout of exercise may not be a potent enough stimulus to alter mitochondrial morphology. Mitochondrial morphological adaptations following longer term exercise protocols were originally demonstrated by Kirkwood *et al*, whereby mitochondria in skeletal muscle adapted to chronic exercise by increasing the extensiveness of the mitochondrial reticulum (57). In response to chronic exercise, our laboratory has demonstrated that both SS and IMF mitochondria respond by increasing mitochondrial volume (49). This suggests that chronic exercise shifts the expression of fission and fusion machinery towards that of enhanced fusion. The alterations in mitochondrial biogenesis are driven, in part, by PGC-1α, a major regulator of mitochondrial biogenesis (37). Mice deficient in PGC-1α have a reduced expression of Mfn2 mRNA (107). Conversely, an upregulation in PGC-1α led to the induction of Mfn2, in a ERRα dependent manner (13). Moreover, Garnier *et al* (33) also found a coordinate increase in mitochondrial biogenesis, specifically PGC-1α, and Mfn2 transcriptional levels. We recently demonstrated that chronic contractile activity upregulates the expression Mfn2 and Opa1, concomitantly with the suppression of the fission protein Drp1 (49). Thus, regular exercise alters the balance of fission and fusion proteins, leading to more reticular mitochondria.

3.3.4. Alterations in mitochondria morphology with diabetes

Diabetes is defined as a group of metabolic disorders, characterized by abnormally elevated plasma glucose concentrations, due to insufficient insulin secretion or abnormal insulin response. The most common form is Type 2 diabetes (T2D), and comprises of approximately 90% of all diabetics cases around the world (1). T2D is associated with insulin resistance and hyperglycemia. The maintenance of normal glucose homeostasis depends on a variety of tissues, such as skeletal muscle, a predominant site of insulin-mediated glucose uptake (23; 24).

Mitochondrial dysfunction is involved in the pathogenesis of diabetes. In particular, skeletal muscle of patients with T2D display a reduction in mitochondrial DNA content (86). These individuals also exhibit decreases in electron transport chain activity, as well as in enzymes of Krebs' cycle,

specifically citrate synthase (53) and succinate oxidase (86). These results support the concept of functional impairments in the mitochondria of T2D patients.

Several mechanisms regulate the reduction in mitochondrial activity in insulin-resistant patients, such as a reduction in mitochondrial content. In this regard, mitochondria from the skeletal muscle of T2D patients have been found to be smaller in size than in muscle from lean individuals (53). The size of mitochondria correlated with insulin sensitivity and glucose disposal rates (53). Upon closer examination the morphology regulatory protein, Mfn2 was found to have a positive correlation with insulin sensitivity (6). T2D patients display reduced Mfn2 mRNA levels relative to their lean counterparts (6). Moreover, the repression of Mfn2 has been shown to reduce glucose oxidation (7). These findings suggest that mitochondrial dynamics are tied to mitochondrial dysfunction and insulin sensitivity.

3.4. Mitochondrially-mediated apoptosis

Apoptosis is the process of programmed cell death that occurs when a cell is no longer required or damaged. Ultrastructural features of apoptosis include DNA fragmentation, plasma membrane blebbing, loss of membrane integrity, cell shrinkage, and chromatin condensation (55). The elimination of cells is a finely controlled process regulated by the activation of pro- and anti-apoptotic proteins. Mitochondrial outer membrane permeability leads to the loss of mitochondrial membrane potential and allows for the release of apoptotic factors, such as cytochrome c into the cytosol. The release of apoptotic proteins from mitochondria is facilitated by specialized pores, termed the mitochondrial permeability transition pore (mtPTP) and the mitochondrial apoptosis-inducing channel (MAC). MACs are composed of oligomerized Bax and/or Bak, and are localized to the outer membrane of mitochondria.

The connection between mitochondrial dynamics and apoptosis is intriguing. During apoptosis the mitochondrial network became fragmented in S. cerevisiae, C. elegans, COS-7 and HeLa cells (30; 31; 51; 62). Perhaps compromised levels of mitochondrial fusion or enhanced levels of fission can potentially sensitize cells to apoptotic stimuli. In support of these finding, the down-regulation of the fission machinery components prevents mitochondrial fragmentation, and reduces apoptotic cell death (31). Conversely, the over-expression of fusion machinery components has been demonstrated to inhibit apoptotic mitochondrial fragmentation, delay Bax/Bak activation, and cytochrome c release (99). The major stores of cytochrome c are sequestered within mitochondrial cristae. Remodeling of the inner membrane, by changes in the shape of the cristae junctions, correlates with the redistribution of cytochrome c (92). The pro-fusion protein, Opa1 has been found to control cristae morphology, whereby the over-expression of Opa1 allows for the maintenance of tight cristae junctions, decreasing cytochrome c release, and thus protecting the cell from apoptosis (32; 61). This suggests that the cellular apoptotic effects are modulated, in part, by the mitochondrial morphology proteins which govern fission and fusion.

3.5. Autophagy

Autophagy involves the sequestering of cytoplasmic material, its encapsulation and eventual digestion through delivery to the lysosome in response to stressful conditions, as a means of removal of damaged or superfluous organelles and proteins (36). Autophagy can promote cell survival and maintain homeostasis, but it can also promote cell death through excessive self-digestion. Selective removal of dysfunctional mitochondria (i.e. mitophagy) is induced as a result of elevated mitochondrial ROS production (91), as well as the dissipation of the mitochondrial membrane potential (85; 102). During this process, the cytosolic E3 ubiquitin ligase, Parkin, is recruited to dysfunctional organelles with reduced membrane potential in a PINK1-dependent manner, flagging them for elimination by ubiquitinating the organelle. Upon encapsulation of the dysfunctional mitochondria, the autophagosome delivers this material to the lysosome for degradation (103).

Mitochondrial morphology is closely integrated with mitophagy. For example, Parkin is an important component of the mitophagy process, and it also plays a critical role in modulating the morphology of mitochondria, such that inhibition of mitochondrial fission has been demonstrated to reduce Parkin-mediated mitophagy (101). Moreover, Mfn1/2 are ubiquitinated in a PINK1/Parkin-dependent manner upon induction of mitophagy (34). Therefore, the PINK1/Parkin pathway is thought to be the intermediate step between mitochondrial remodeling and mitophagy. Inhibition of the fission machinery components, Drp1 and Fis1, and over-expression of the pro-fusion protein, Opa1 all suppress the

degree of mitophagy (102). For mitophagy to occur, dysfunctional organelles must be segregated and processed into smaller, fragmented components in order to be engulfed by autophagosomes (102). This alteration in mitochondrial shape involves fission and fusion machinery components. Therefore, there is a close interaction between the turnover of mitochondria via mitophagy, and the expression and function of fission/fusion machinery components, which ultimately regulate the level of mitochondrial fragmentation.

3.6. Unfolded protein response

The endoplasmic reticulum (ER) and mitochondria are exposed to newly synthesized proteins and are responsible for their proper folding and assembly. Homeostasis is achieved by balancing the nascent protein load with the folding capacity of the organelle. When the ability to do so is compromised, ER and/or mitochondrial stress ensues. The adaptive responses to this imbalance, termed the ER-unfolded protein response (UPR) and the mitochondrial UPR (mtUPR), coincide with the upregulation of molecular chaperones allowing for the increased folding capacity of the cell. If the cellular stress is too extensive and cannot be alleviated, the UPR activates cell destructive responses including apoptosis and the mitophagy pathway (80).

ER stress triggers three ER transmembrane proteins, inositol requiring 1 (IRE1), double stranded RNA activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6). Once these stress sensors become activated they initiate the UPR by effecting downstream targets. IRE1, PERK, and ATF6 are maintained in an inactive state through interactions with the ER chaperone protein Binding Ig Protein (BiP). In response to ER stress, PERK is liberated from BiP, thus allowing for its autophosphorylation and activation. Subsequently, PERK phosphorylates eukaryotic initiation factor 2 (eIF2α), leading to inhibition of global protein translation (39; 93). An additional downstream target of PERK includes C/EBP-homologous protein (CHOP) (38). CHOP is a transcription factor that binds to and activates the promoter of stress response genes, such as c- Jun N-terminal Kinase (JNK) (46). As such, it is capable of propagating the UPR signal. Furthermore, it is also known that CHOP activates the transcription of mtUPR chaperone genes, such as mtHSP60 (80). Ngoh *et al* (72) discovered that Mfn2 regulated the ER-UPR, and was upregulated in response to ER stress. Munoz *et al* (69) built on this knowledge and found that Mfn2 was also an upstream regulator of PERK. Thus, growing evidence is suggesting a connection between mitochondrial morphology protein expression and the UPR however more work is required to better understand its influence on mitochondrial morphology.

In addition to the protective effects of the ER-UPR, the mechanisms governing the protein folding environment within mitochondria are beginning to be elucidated. The majority of mitochondrially-destined proteins are encoded by the nucleus and imported into the mitochondria in an unfolded state. Failure of these proteins to fold properly can result in ROS accumulation (10). ROS generation, in response to mitochondrial-specific stress, activates general control nonrepressed 2 (GCN2), which subsequently phosphorylates eIF2α to reduce the translation of proteins (10). A complementary pathway to that of GCN2 and eIF2α involves activating transcription factor 4 (ATF4). ATF4 can upregulate the transcription factor CHOP, thereby allowing for the amplification of the signal initiated by the original stress (5; 41). In skeletal muscle subject to conditions of chronic muscle disuse (2) and aging (15), an increased production of ROS from mitochondria occurs, and this coincides with an increased incidence of mitochondrial fragmentation (49). Silencing of the UPR protein PERK restores mitochondrial morphology towards a reticular structure (69). Although the precise mechanism is not yet clear, recent experiments by Nargund *et al* have found that mitochondrial fission genes are involved in the mtUPR in *C. elegans* (71). The notion that cellular stressors, such as ROS, can activate the ER-UPR and mtUPR is intriguing, especially since we are beginning to discover the involvement of these UPR proteins in mitochondrial functions.

4. SUMMARY

The maintenance of mitochondrial morphology depends on reaching an equilibrium between fission and fusion. Fusion involves the mixing of mitochondrial material, whereas the fission process divides the organelle into smaller components. Disruptions in either of these opposing events can lead to developmental defects and disease (108), suggesting that proper maintenance of mitochondrial morphology is critical for normal cell function. Skeletal muscle organelle morphology has been demonstrated to be altered under various

disease conditions, including diabetes (6; 53), denervation (49), as well as during development (8), aging (47), and exercise (57). Recent evidence indicates that proteins participating in mitochondrial morphology are also involved in the regulatory processes of apoptosis, autophagy, and the unfolded protein responses. Therefore, both fusion and fission processes are essential for normal cellular health. Further knowledge into the molecular signaling mechanisms involved in the control of mitochondrial dynamics is warranted to help understand novel mechanisms underlying the role of mitochondria in muscle health and disease.

5. ACKNOWLEDGEMENTS

This work was supported by a Natural Science and Engineering Research Council of Canada (NSERC) grant to D.A. Hood. D.A. Hood holds a Canada Research Chair in Cell Physiology.

6. REFERENCES

1. World Health Organization (Online) (2011)
2. Adhihetty PJ, O'Leary MF, Chabi B, Wicks KL and Hood DA: Effect of denervation on mitochondrially mediated apoptosis in skeletal muscle. J Appl Physiol 102, 1143-1151 (2007)
DOI: 10.1152/japplphysiol.00768.2006
3. Akimoto T, Pohnert SC, Li P, Zhang M, Gumbs C, Rosenberg PB, Williams RS and Yan Z: Exercise stimulates Pgc-1alpha transcription in skeletal muscle through activation of the p38 MAPK pathway. J Biol Chem 280, 19587-19593 (2005)
DOI: 10.1074/jbc.M408862200
4. Altmann R. Die Elementarorganismen Und Ihre Beziehungen Zu Den Zellen. Leipzig: Veit & comp, (1890)
5. B'chir W, Maurin AC, Carraro V, Averous J, Jousse C, Muranishi Y, Parry L, Stepien G, Fafournoux P and Bruhat A: The eIF2alpha/ATF4 pathway is essential for stress-induced autophagy gene expression. Nucleic Acids Res 41, 7683-7699 (2013)
DOI: 10.1093/nar/gkt563
6. Bach D, Naon D, Pich S, Soriano FX, Vega N, Rieusset J, Laville M, Guillet C, Boirie Y, Wallberg-Henriksson H, Manco M, Calvani M, Castagneto M, Palacin M, Mingrone G, Zierath JR, Vidal H and Zorzano A: Expression of Mfn2, the Charcot-Marie-Tooth neuropathy type 2A gene, in human skeletal muscle: effects of type 2 diabetes, obesity, weight loss, and the regulatory role of tumor necrosis factor alpha and interleukin-6. Diabetes 54, 2685-2693 (2005)
DOI: 10.2337/diabetes.54.9.2685
7. Bach D, Pich S, Soriano FX, Vega N, Baumgartner B, Oriola J, Daugaard JR, Lloberas J, Camps M, Zierath JR, Rabasa-Lhoret R, Wallberg-Henriksson H, Laville M, Palacin M, Vidal H, Rivera F, Brand M and Zorzano A: Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism. A novel regulatory mechanism altered in obesity. J Biol Chem 278, 17190-17197 (2003)
DOI: 10.1074/jbc.M212754200
8. Bakeeva LE, Chentsov YS and Skulachev VP: Ontogenesis of mitochondrial reticulum in rat diaphragm muscle. Eur J Cell Biol 25, 175-181 (1981)
9. Bakeeva LE, Chentsov Y and Skulachev VP: Mitochondrial framework (reticulum mitochondriale) in rat diaphragm muscle. Biochim Biophys Acta 501, 349-369 (1978)
DOI: 10.1016/0005-2728(78)90104-4
10. Baker BM, Nargund AM, Sun T and Haynes CM: Protective coupling of mitochondrial function and protein synthesis via the eIF2alpha kinase GCN-2. PLoS Genet 8, e1002760 (2012)
DOI: 10.1371/journal.pgen.1002760
11. Balaban RS, Nemoto S and Finkel T: Mitochondria, oxidants, and aging. Cell 120, 483-495 (2005)
DOI: 10.1016/j.cell.2005.02.001
12. Bleazard W, McCaffery JM, King EJ, Bale S, Mozdy A, Tieu Q, Nunnari J and Shaw

JM: The dynamin-related GTPase Dnm1 regulates mitochondrial fission in yeast. Nat Cell Biol, 298-304 (1999)

13. Cartoni R, Leger B, Hock MB, Praz M, Crettenand A, Pich S, Ziltener JL, Luthi F, Deriaz O, Zorzano A, Gobelet C, Kralli A and Russell AP: Mitofusins 1/2 and ERRalpha expression are increased in human skeletal muscle after physical exercise. J Physiol 567, 349-358 (2005) DOI: 10.1113/jphysiol.2005.092031
14. Cereghetti GM, Stangherlin A, Martins de BO, Chang CR, Blackstone C, Bernardi P and Scorrano L: Dephosphorylation by calcineurin regulates translocation of Drp1 to mitochondria. Proc Natl Acad Sci U S A 105, 15803-15808 (2008) DOI: 10.1073/pnas.0808249105
15. Chabi B, Ljubicic V, Menzies KJ, Huang JH, Saleem A and Hood DA: Mitochondrial function and apoptotic susceptibility in aging skeletal muscle. Aging Cell 7, 2-12 (2008) DOI: 10.1111/j.1474-9726.2007.00347.x
16. Chang CR and Blackstone:C. Cyclic AMP-dependent protein kinase phosphorylation of Drp1 regulates its GTPase activity and mitochondrial morphology. J Biol Chem 282, 21583-21587 (2007) DOI: 10.1074/jbc.C700083200
17. Chen H, Chomyn A and Chan DC: Disruption of fusion results in mitochondrial heterogeneity and dysfunction. J Biol Chem 280, 26185-26192 (2005) DOI: 10.1074/jbc.M503062200
18. Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE and Chan DC: Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. J Cell Biol 160, 189-200 (2003) DOI: 10.1083/jcb.200211046
19. Chen H, Vermulst M, Wang YE, Chomyn A, Prolla TA, McCaffery JM and Chan DC: Mitochondrial fusion is required for mtDNA stability in skeletal muscle and tolerance of mtDNA mutations. Cell 141, 280-289 (2010) DOI: 10.1016/j.cell.2010.02.026
20. Cho SG, Du Q, Huang S and Dong Z: Drp1 dephosphorylation in ATP depletion-induced mitochondrial injury and tubular cell apoptosis. Am J Physiol Renal Physiol 299, F199-F206 (2010) DOI: 10.1152/ajprenal.00716.2009
21. Cipolat S, Martins de BO, Dal ZB and Scorrano L.: OPA1 requires mitofusin 1 to promote mitochondrial fusion. Proc Natl Acad Sci U S A 101, 15927-15932 (2004) DOI: 10.1073/pnas.0407043101
22. Cribbs JT and Strack S: Reversible phosphorylation of Drp1 by cyclic AMP-dependent protein kinase and calcineurin regulates mitochondrial fission and cell death. EMBO Rep 8, 939-944 (2007) DOI: 10.1038/sj.embor.7401062
23. DeFronzo RA, Gunnarsson R, Bjorkman O, Olsson M and Wahren J: Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. J Clin Invest 76, 149-155 (1985) DOI: 10.1172/JCI111938
24. DeFronzo RA and Tripathy D: Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. Diabetes Care 32 Suppl 2, S157-S163 (2009) DOI: 10.2337/dc09-S302
25. Delettre C, Griffoin JM, Kaplan J, Dollfus H, Lorenz B, Faivre L, Lenaers G, Belenguer P and Hamel CP: Mutation spectrum and splicing variants in the OPA1 gene. Hum Genet 109, 584-591 (2001) DOI: 10.1007/s00439-001-0633-y
26. Dirks AJ and Leeuwenburgh C: Aging and lifelong calorie restriction result in adaptations of skeletal muscle apoptosis repressor, apoptosis-inducing factor, X-linked inhibitor of apoptosis, caspase-3, and caspase-12. Free Radic Biol Med 36, 27-39 (2004) DOI: 10.1016/j.freeradbiomed.2003.10.003
27. Dohm JA, Lee SJ, Hardwick JM, Hill RB and Gittis AG: Cytosolic domain of the

human mitochondrial fission protein fis1 adopts a TPR fold. Proteins 54, 153-156 (2004)
DOI: 10.1002/prot.10524

28. Ehses S, Raschke I, Mancuso G, Bernacchia A, Geimer S, Tondera D, Martinou JC, Westermann B, Rugarli EI and Langer T: Regulation of OPA1 processing and mitochondrial fusion by m-AAA protease isoenzymes and OMA1. J Cell Biol 187, 1023-1036 (2009)
DOI: 10.1083/jcb.200906084

29. Fan X, Hussien R and Brooks GA: H2O2-induced mitochondrial fragmentation in C2C12 myocytes. Free Radic Biol Med 49, 1646-1654, (2010).
DOI: 10.1016/j.freeradbiomed.2010.08.024

30. Fannjiang Y, Cheng WC, Lee SJ, Qi B, Pevsner J, McCaffery JM, Hill RB, Basanez G and Hardwick JM: Mitochondrial fission proteins regulate programmed cell death in yeast. Genes Dev 18, 2785-2797 (2004)
DOI: 10.1101/gad.1247904

31. Frank S, Gaume B, Bergmann-Leitner ES, Leitner WW, Robert EG, Catez F, Smith CL and Youle RJ: The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. Dev Cell 1, 515-525 (2001)
DOI: 10.1016/S1534-5807(01)00055-7

32. Frezza C, Cipolat S, Martins de BO, Micaroni M, Beznoussenko GV, Rudka T, Bartoli D, Polishuck RS, Danial NN, De SB and Scorrano L: OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. Cell 126, 177-189 (2006)
DOI: 10.1016/j.cell.2006.06.025

33. Garnier A, Fortin D, Zoll J, N'Guessan B, Mettauer B, Lampert E, Veksler V and Ventura-Clapier R: Coordinated changes in mitochondrial function and biogenesis in healthy and diseased human skeletal muscle. FASEB J 19, 43-52 (2005)
DOI: 10.1096/fj.04-2173com

34. Gegg ME, Cooper JM, Chau KY, Rojo M, Schapira AH and Taanman JW: Mitofusin 1 and mitofusin 2 are ubiquitinated in a PINK1/parkin-dependent manner upon induction of mitophagy. Hum Mol Genet 19, 4861-4870 (2010)
DOI: 10.1093/hmg/ddq419

35. Gordon JW, Rungi AA, Inagaki H and Hood DA: Effects of contractile activity on mitochondrial transcription factor A expression in skeletal muscle. J Appl Physiol 90, 389-396 (2001)

36. Gustafsson AB and Gottlieb RA: Recycle or die: the role of autophagy in cardioprotection. J Mol Cell Cardiol 44, 654-661 (2008)
DOI: 10.1016/j.yjmcc.2008.01.010

37. Handschin C and Spiegelman BM: Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. Endocr Rev 27, 728-735 (2006)
DOI: 10.1210/er.2006-0037

38. Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, Schapira M and Ron D: Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol Cell 6, 1099-1108 (2000)
DOI: 10.1016/S1097-2765(00)00108-8

39. Harding HP, Zhang Y and Ron D: Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature 397, 271-274 (1999)
DOI: 10.1038/16729

40. Harman D: Aging: a theory based on free radical and radiation chemistry. J Gerontol 11, 298-300 (1956)
DOI: 10.1093/geronj/11.3.298

41. Haynes CM, Fiorese CJ and Lin YF: Evaluating and responding to mitochondrial dysfunction: the mitochondrial unfolded-protein response and beyond. Trends Cell Biol 23, 311-318 (2013)
DOI: 10.1016/j.tcb.2013.02.002

42. Herlan M, Vogel F, Bornhovd C, Neupert W and Reichert AS: Processing of Mgm1 by the rhomboid-type protease Pcp1 is required for maintenance of mitochondrial

morphology and of mitochondrial DNA. J Biol Chem 278, 27781-27788 (2003)
DOI: 10.1074/jbc.M211311200

43. Holloszy JO: Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. J Biol Chem 242, 2278-2282 (1967)

44. Hood DA: Invited Review: contractile activity-induced mitochondrial biogenesis in skeletal muscle. J Appl Physiol (1985) 90, 1137-1157 (2001)

45. Hood DA, Uguccioni G, Vainshtein A and D'souza D: Mechanisms of exercise-induced mitochondrial biogenesis in skeletal muscle: implications for health and disease. Compr Physiol 1, 1119-1134 (2011)

46. Horibe T and Hoogenraad NJ: The chop gene contains an element for the positive regulation of the mitochondrial unfolded protein response. PLoS One 2, e835 (2007)
DOI: 10.1371/journal.pone.0000835

47. Huang JH, Joseph AM, Ljubicic V, Iqbal S and Hood DA" Effect of age on the processing and import of matrix-destined mitochondrial proteins in skeletal muscle. J Gerontol A Biol Sci Med Sci 65, 138-146 (2010)
DOI: 10.1093/gerona/glp201

48. Iqbal S and Hood DA: Oxidative stress-induced mitochondrial fragmentation and movement in skeletal muscle cells. Am J Physiol Cell Physiol 2014. In press.
DOI: 10.1152/ajpcell.00017.2014

49. Iqbal S, Ostojic O, Singh K, Joseph AM and Hood DA: Expression of mitochondrial fission and fusion regulatory proteins in skeletal muscle during chronic use and disuse. Muscle Nerve 48, 963-970 (2013)
DOI: 10.1002/mus.23838

50. Ishihara N, Eura Y and Mihara K: Mitofusin 1 and 2 play distinct roles in mitochondrial fusion reactions via GTPase activity. J Cell Sci 117, 6535-6546, 2004
DOI: 10.1242/jcs.01565

51. Jagasia R, Grote P, Westermann B and Conradt B: DRP-1-mediated mitochondrial fragmentation during EGL-1-induced cell death in C. elegans. Nature 433, 754-760 (2005)
DOI: 10.1038/nature03316

52. Jerusalem F, Engel AG and Peterson HA: Human muscle fiber fine structure: morphometric data on controls. Neurology 25, 127-134 (1975)
DOI: 10.1212/WNL.25.2.127

53. Kelley DE, He J, Menshikova EV and Ritov VB: Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes 51, 2944-2950 (2002)
DOI: 10.2337/diabetes.51.10.2944

54. Kemper C, Habib SJ, Engl G, Heckmeyer P, Dimmer KS and Rapaport D: Integration of tail-anchored proteins into the mitochondrial outer membrane does not require any known import components. J Cell Sci 121, 1990-1998 (2008)
DOI: 10.1242/jcs.024034

55. Kerr JF, Wyllie AH and Currie AR: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26,239-257 (1972)
DOI: 10.1038/bjc.1972.33

56. Kirkwood SP, Munn EA and Brooks GA: Mitochondrial reticulum in limb skeletal muscle. Am J Physiol 251, C395-C402 (1986)

57. Kirkwood SP, Packer L and Brooks GA: Effects of endurance training on a mitochondrial reticulum in limb skeletal muscle. Arch Biochem Biophys 255, 80-88 (1987)
DOI: 10.1016/0003-9861(87)90296-7

58. Koshiba T, Detmer SA, Kaiser JT, Chen H, McCaffery JM and Chan DC: Structural basis of mitochondrial tethering by mitofusin complexes. Science 305, 858-862 (2004)
DOI: 10.1126/science.1099793

59. Krieger DA, Tate CA, Millin-Wood J and Booth FW: Populations of rat skeletal muscle mitochondria after exercise and

immobilization. J Appl Physiol 48, 23-28 (1980)

60. Lai RY, Ljubicic V, D'souza D and Hood DA: Effect of chronic contractile activity on mRNA stability in skeletal muscle. Am J Physiol Cell Physiol 299, C155-C163 (2010)
DOI: 10.1152/ajpcell.00523.2009

61. Landes T, Emorine LJ, Courilleau D, Rojo M, Belenguer P and rnaune-Pelloquin L: The BH3-only Bnip3 binds to the dynamin Opa1 to promote mitochondrial fragmentation and apoptosis by distinct mechanisms. EMBO Rep 11, 459-465 (2010)
DOI: 10.1038/embor.2010.50

62. Lee YJ, Jeong SY, Karbowski M, Smith CL and Youle RJ: Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. Mol Biol Cell 15, 5001-5011 (2004)
DOI: 10.1091/mbc.E04-04-0294

63. Lewis MR and Lewis WH: Mitochondria (and other cytoplasmic structures) in tissue cultures. Am J Anat 17, 339-401, (1915).
DOI: 10.1002/aja.1000170304

64. Liesa M, Palacin M and Zorzano A: Mitochondrial dynamics in mammalian health and disease. Physiol Rev 89, 799-845 (2009)
DOI: 10.1152/physrev.00030.2008

65. Lopez ME, Van Zeeland NL, Dahl DB, Weindruch R and Aiken JM: Cellular phenotypes of age-associated skeletal muscle mitochondrial abnormalities in rhesus monkeys. Mutat Res 452, 123-138 (2000)
DOI: 10.1016/S0027-5107(00)00059-2

66. Mansouri A, Muller FL, Liu Y, Ng R, Faulkner J, Hamilton M, Richardson A, Huang TT, Epstein CJ and Van RH: Alterations in mitochondrial function, hydrogen peroxide release and oxidative damage in mouse hind-limb skeletal muscle during aging. Mech Ageing Dev 127, 298-306 (2006)
DOI: 10.1016/j.mad.2005.11.004

67. Midrio M: The denervated muscle: facts and hypotheses. A historical review. Eur J Appl Physiol 98, 1-21 (2006)
DOI: 10.1007/s00421-006-0256-z

68. Miledi R and Slater CR. Some mitochondrial changes in denervated muscle. J Cell Sci 3: 49-54 (1968)

69. Munoz JP, Ivanova S, Sanchez-Wandelmer J, Martinez-Cristobal P, Noguera E, Sancho A, az-Ramos A, Hernandez-Alvarez MI, Sebastian D, Mauvezin C, Palacin M and Zorzano A: Mfn2 modulates the UPR and mitochondrial function via repression of PERK. EMBO J 32, 2348-2361 (2013)
DOI: 10.1038/emboj.2013.168

70. Musacchia XJ, Steffen JM and Fell RD: Disuse atrophy of skeletal muscle: animal models. Exerc Sport Sci Rev 16, 61-87 (1988)
DOI: 10.1249/00003677-198800160-00005

71. Nargund AM, Pellegrino MW, Fiorese CJ, Baker BM and Haynes CM: Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. Science 337, 587-590 (2012)
DOI: 10.1126/science.1223560

72. Ngoh GA, Papanicolaou KN and Walsh K: Loss of mitofusin 2 promotes endoplasmic reticulum stress. J Biol Chem 287, 20321-20332 (2012)
DOI: 10.1074/jbc.M112.359174

73. O'Leary MF and Hood DA: Effect of prior chronic contractile activity on mitochondrial function and apoptotic protein expression in denervated muscle. J Appl Physiol 105, 114-120 (2008)
DOI: 10.1152/japplphysiol.00724.2007

74. Ogata T and Yamasaki Y: Scanning electron-microscopic studies on the three-dimensional structure of mitochondria in the mammalian red, white and intermediate muscle fibers. Cell Tissue Res 241, 251-256 (1985)
DOI: 10.1007/BF00217168

75. Olichon A, Baricault L, Gas N, Guillou E, Valette A, Belenguer P and Lenaers

G: Loss of OPA1 perturbates the mitochondrial inner membrane structure and integrity, leading to cytochrome c release and apoptosis. J Biol Chem 278, 7743-7746 (2003)
DOI: 10.1074/jbc.C200677200

76. Olichon A, Emorine LJ, Descoins E, Pelloquin L, Brichese L, Gas N, Guillou E, Delettre C, Valette A, Hamel CP, Ducommun B, Lenaers G and Belenguer P: The human dynamin-related protein OPA1 is anchored to the mitochondrial inner membrane facing the inter-membrane space. FEBS Lett 523, 171-176 (2002)
DOI: 10.1016/S0014-5793(02)02985-X

77. Ono T, Isobe K, Nakada K and Hayashi JI: Human cells are protected from mitochondrial dysfunction by complementation of DNA products in fused mitochondria. Nat Genet 28, 272-275 (2001)
DOI: 10.1038/90116

78. Palmer JW, Tandler B and Hoppel CL: Biochemical properties of subsarcolemmal and interfibrillar mitochondria isolated from rat cardiac muscle. J Biol Chem 252, 8731-8739 (1977)

79. Parone PA, Da CS, Tondera D, Mattenberger Y, James DI, Maechler P, Barja F and Martinou JC: Preventing mitochondrial fission impairs mitochondrial function and leads to loss of mitochondrial DNA. PLoS One 3, e3257 (2008)
DOI: 10.1371/journal.pone.0003257

80. Pellegrino MW, Nargund AM and Haynes CM: Signaling the mitochondrial unfolded protein response. Biochim Biophys Acta 1833, 410-416 (2013)
DOI: 10.1016/j.bbamcr.2012.02.019

81. Picard M, Gentil BJ, McManus MJ, White K, St LK, Gartside SE, Wallace DC and Turnbull DM: Acute exercise remodels mitochondrial membrane interactions in mouse skeletal muscle. J Appl Physiol 115, 1562-1571 (2013)
DOI: 10.1152/japplphysiol.00819.2013

82. Picard M, White K and Turnbull DM: Mitochondrial morphology, topology and membrane interactions in skeletal muscle: A quantitative three-dimensional electron microscopy study. J Appl Physiol 114, 161-171 (2013)
DOI: 10.1152/japplphysiol.01096.2012

83. Pilegaard H, Saltin B and Neufer PD: Exercise induces transient transcriptional activation of the PGC-1alpha gene in human skeletal muscle. J Physiol 546, 851-858 (2003)
DOI: 10.1113/jphysiol.2002.034850

84. Poggi P, Marchetti C and Scelsi R: Automatic morphometric analysis of skeletal muscle fibers in the aging man. Anat Rec 217, 30-34 (1987)
DOI: 10.1002/ar.1092170106

85. Priault M, Salin B, Schaeffer J, Vallette FM, di Rago JP and Martinou JC: Impairing the bioenergetic status and the biogenesis of mitochondria triggers mitophagy in yeast. Cell Death Differ 12, 1613-1621 (2005)
DOI: 10.1038/sj.cdd.4401697

86. Ritov VB, Menshikova EV, He J, Ferrell RE, Goodpaster BH and Kelley DE: Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. Diabetes 54, 8-14 (2005)
DOI: 10.2337/diabetes.54.1.8

87. Rojo M, Legros F, Chateau D and Lombes A: Membrane topology and mitochondrial targeting of mitofusins, ubiquitous mammalian homologs of the transmembrane GTPase Fzo. J Cell Sci 115, 1663-1674 (2002)

88. Romanello V, Guadagnin E, Gomes L, Roder I, Sandri C, Petersen Y, Milan G, Masiero E, Del PP, Foretz M, Scorrano L, Rudolf R and Sandri M: Mitochondrial fission and remodelling contributes to muscle atrophy. EMBO J 29, 1774-1785 (2010)
DOI: 10.1038/emboj.2010.60

89. Santel A, Frank S, Gaume B, Herrler M, Youle RJ and Fuller MT: Mitofusin-1 protein is a generally expressed mediator

of mitochondrial fusion in mammalian cells. J Cell Sci 116, 2763-2774 (2003)
DOI: 10.1242/jcs.00479

90. Santel A and Fuller MT: Control of mitochondrial morphology by a human mitofusin. J Cell Sci 114, 867-874 (2001)

91. Scherz-Shouval R, Shvets E, Fass E, Shorer H, Gil L and Elazar Z: Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. EMBO J 26, 1749-1760 (2007)
DOI: 10.1038/sj.emboj.7601623

92. Scorrano L, Ashiya M, Buttle K, Weiler S, Oakes SA, Mannella CA and Korsmeyer SJ: A distinct pathway remodels mitochondrial cristae and mobilizes cytochrome c during apoptosis. Dev Cell 2, 55-67 (2002)
DOI: 10.1016/S1534-5807(01)00116-2

93. Seo HY, Kim YD, Lee KM, Min AK, Kim MK, Kim HS, Won KC, Park JY, Lee KU, Choi HS, Park KG and Lee IK: Endoplasmic reticulum stress-induced activation of activating transcription factor 6 decreases insulin gene expression via up-regulation of orphan nuclear receptor small heterodimer partner. Endocrinology 149, 3832-3841 (2008)
DOI: 10.1210/en.2008-0015

94. Serasinghe MN and Yoon Y: The mitochondrial outer membrane protein hFis1 regulates mitochondrial morphology and fission through self-interaction. Exp Cell Res 314, 3494-3507 (2008)
DOI: 10.1016/j.yexcr.2008.09.009

95. Singh K and Hood DA: Effect of denervation-induced muscle disuse on mitochondrial protein import. Am J Physiol Cell Physiol 300, C138-C145 (2011)
DOI: 10.1152/ajpcell.00181.2010

96. Smirnova E, Shurland DL, Ryazantsev SN and van der Bliek AM: A human dynamin-related protein controls the distribution of mitochondria. J Cell Biol 143, 351-358 (1998)
DOI: 10.1083/jcb.143.2.351

97. Stojanovski D, Koutsopoulos OS, Okamoto K and Ryan MT: Levels of human Fis1 at the mitochondrial outer membrane regulate mitochondrial morphology. J Cell Sci 117, 1201-1210 (2004)
DOI: 10.1242/jcs.01058

98. Strack S and Cribbs JT: Allosteric modulation of Drp1 mechanoenzyme assembly and mitochondrial fission by the variable domain. J Biol Chem 287, 10990-11001 (2012)
DOI: 10.1074/jbc.M112.342105

99. Sugioka R, Shimizu S and Tsujimoto Y: Fzo1, a protein involved in mitochondrial fusion, inhibits apoptosis. J Biol Chem 279, 52726-52734 (2004)
DOI: 10.1074/jbc.M408910200

100. Suzuki M, Jeong SY, Karbowski M, Youle RJ and Tjandra N: The solution structure of human mitochondria fission protein Fis1 reveals a novel TPR-like helix bundle. J Mol Biol 334, 445-458 (2003)
DOI: 10.1016/j.jmb.2003.09.064

101. Tanaka A, Cleland MM, Xu S, Narendra DP, Suen DF, Karbowski M and Youle RJ: Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. J Cell Biol 191, 1367-1380 (2010)
DOI: 10.1083/jcb.201007013

102. Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, Alroy J, Wu M, Py BF, Yuan J, Deeney JT, Corkey BE and Shirihai OS: Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. EMBO J 27, 433-446 (2008)
DOI: 10.1038/sj.emboj.7601963

103. Uchiyama Y: Autophagic cell death and its execution by lysosomal cathepsins. Arch Histol Cytol 64, 233-246 (2001)
DOI: 10.1679/aohc.64.233

104. Wanagat J, Cao Z, Pathare P and Aiken JM: Mitochondrial DNA deletion mutations colocalize with segmental electron transport system abnormalities, muscle fiber atrophy, fiber splitting, and oxidative

damage in sarcopenia. FASEB J 15, 322-332 (2001)
DOI: 10.1096/fj.00-0320com

105. Wang JX, Jiao JQ, Li Q, Long B, Wang K, Liu JP, Li YR and Li PF: miR-499 regulates mitochondrial dynamics by targeting calcineurin and dynamin-related protein-1. Nat Med 17, 71-78 (2011)
DOI: 10.1038/nm.2282

106. Wicks KL and Hood DA: Mitochondrial adaptations in denervated muscle: relationship to muscle performance. Am J Physiol 260, C841-C850 (1991)

107. Zechner C, Lai L, Zechner JF, Geng T, Yan Z, Rumsey JW, Collia D, Chen Z, Wozniak DF, Leone TC and Kelly DP: Total skeletal muscle PGC-1 deficiency uncouples mitochondrial derangements from fiber type determination and insulin sensitivity. Cell Metab 12, 633-642 (2010)
DOI: 10.1016/j.cmet.2010.11.008

108. Zuchner S, Mersiyanova IV, Muglia M, Bissar-Tadmouri N, Rochelle J, Dadali EL, Zappia M, Nelis E, Patitucci A, Senderek J, Parman Y, Evgrafov O, Jonghe PD, Takahashi Y, Tsuji S, Pericak-Vance MA, Quattrone A, Battaloglu E, Polyakov AV, Timmerman V, Schroder JM and Vance JM: Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. Nat Genet 36, 449-451 (2004)
DOI: 10.1038/ng1341

Abbreviations: ATF4: activating transcription factor 4, ATF6: activating transcription factor 6, ATG7: autophagy related protein 7, ATP: adenosine triphosphate, Bak: BCL2-antagonist/killer 1 , Bax: Bcl-2 associated X protein, BiP:binding Ig protein, C2C12 Mouse myoblast cell line, *C. elegans Caenorhabditis elegans*, CHOP: C/EBP-homologous protein, COS-7: cell line from African green monkey kidney fibroblasts, COX: cytochrome c oxidase, CS: citrate synthase, C-terminal carboxyl-terminus, DNA:deoxyribonucleic nucleic acid, Drp1: dynamin-related protein 1, eIF2a: eukaryotic initiation factor 2a, ER: endoplasmic reticulum, ERRa: estrogen-related receptor a, Fis1: fission 1, GCN2: general control nonrepressed 2, GTP: guanosine triphosphate, HeLa Henrietta Lacks' cervical cancer cell line, HSP: heat shock protein, IMM: inner mitochondrial membrane, IMF:intermyofibrillar , IRE: inositol requiring 1, JNK: c-Jun N-terminal kinase, LC3 light chain protein 3, MAC mitochondrial apoptosis-inducing channel, Mfn1 mitofusin 1, Mfn2 mitofusin 2, Mgm1 Mitochondrial genome maintenance protein 1, l-Mgm1: large isoform Mitochondrial genome maintenance protein 1, s-Mgm1 short isoform Mitochondrial genome maintenance protein 1, mt: mitochondria, mtDNA:mitochondrial DNA, mtHSP60:mitochondrial heat shock protein 60, mtPTP: mitochondrial permeability transition pore, MHC: myosin heavy chain, NRF-1 nuclear respiratory factor-1, NRF-2:nuclear respiratory factor-2, N-terminal nitrogen associated terminal of protein, NUGEMPs: nuclear genes encoding mitochondrial proteins, O2 oxygen, Opa1: optic atrophy 1, OMM: outer mitochondrial membrane, P-Drp1: phosphorylated dynamin-related protein 1, PERK: PKR-like endoplasmic reticulum kinase, PGC-1a: peroxisome proliferator activated receptor gamma coactivator 1 alpha, PINK1: PTEN-induced putative kinase 1, PKA: cyclic AMP-dependent protein kinase, ROS: reactive oxygen species, S. cervisiae: Saccharomyces cerevisiae, Ser: Serine, SDH: succinate dehydrogenase, SS: subsarcolemmal , T2D: type 2 diabetes , Tfam: transcription factor, TTX: tetrodoxin, ULK1: unc-51-like kinase 1, UPR: unfolded protein response

Key Words: Mitochondria, Mitochondrial Morphology, Fission, Fusion, Review

Send correspondence to: David A. Hood, School of Kinesiology and Health Science, York University, Toronto, Ontario, M3J 1P3, Canada, Tel: 416-736-2100 ext: 66640, Fax: 416-736-5698, E-mail: dhood@yorku.ca