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Emerging roles of ER stress and unfolded protein response pathways in skeletal muscle health and disease

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Abstract

Skeletal muscle is the most abundant tissue in the human body and can adapt its mass as a consequence of physical activity, metabolism, growth factors, and disease conditions. Skeletal muscle contains an extensive network of endoplasmic reticulum (ER), called sarcoplasmic reticulum, which plays an important role in the regulation of proteostasis and calcium homeostasis. In many cell types, environmental and genetic factors that disrupt ER function cause an accumulation of misfolded and unfolded proteins in the ER lumen that ultimately leads to ER stress. To alleviate the stress and restore homeostasis, the ER activates a signaling network called the unfolded protein response (UPR). The UPR has three arms, which regulate protein synthesis and expression of many ER chaperone and regulatory proteins. However, the role of individual UPR pathways in skeletal muscle has just begun to be investigated. Recent studies suggest that UPR pathways play pivotal roles in muscle stem cell homeostasis, myogenic differentiation, and regeneration of injured skeletal muscle. Moreover, markers of ER stress and the UPR are activated in skeletal muscle in diverse conditions such as exercise, denervation, starvation, high fat diet, cancer cachexia, and aging. Accumulating evidence also suggests that ER stress may have important roles in the pathogenesis of inflammatory myopathies and genetic muscle disorders. The purpose of this review article is to discuss the role and potential mechanisms by which ER stress and the individual arms of the UPR regulate skeletal muscle formation, plasticity, and function in various physiological and pathophysiological conditions.

Keywords

Skeletal muscle; Atrophy; Myogenesis; ER Stress; UPR

Introduction

Skeletal muscle is one of the most dynamic and highly plastic tissues of the human body that is essential for posture, locomotion, and breathing. Skeletal muscle contains 50-75% of all

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the body's proteins. It is also one of the most important sites for the control of whole body metabolism. Skeletal muscle utilizes multiple pathways that regulate cell and protein content (Bonaldo and Sandri, 2013). Loss of skeletal muscle mass, commonly referred to as muscle atrophy, is associated with a wide array of catabolic conditions and disease states such as: aging, immobilization, denervation, cancer, HIV, chronic heart failure, burn injury, chronic kidney disease, etc. (Li et al., 2008). In addition, genetic muscle disorders, such as muscular dystrophy, are characterized by degeneration of myofibers and replacement with fibrotic tissue rendering nonfunctional muscle and eventually leading to death as a result of respiratory failure (Shin et al., 2013). Thus, the development of pharmacological therapies that can preserve or restore skeletal muscle mass would be highly beneficial to several clinically relevant populations. However, in order to develop such therapies, a comprehensive understanding of the molecular mechanisms that regulate skeletal muscle mass is a prerequisite.

The endoplasmic reticulum (ER) is a membrane-bound organelle in mammalian cells that is responsible for proper folding, processing, and trafficking of proteins. The ER also plays an important role in calcium homeostasis of the cell. Accumulation of misfolded proteins, viral infection, imbalances in calcium level, and many other perturbations that disrupt cell homeostasis lead to ER stress (Isler et al., 2005; Pyrko et al., 2007; Welihinda et al., 1999). The ER deals with such stress through initiating the unfolded protein response (UPR) that is mediated by three ER transmembrane sensors: RNA-dependent protein kinase-like ER eukaryotic translation initiation factor 2 alpha kinase (PERK), inositol-requiring protein 1 (IRE1), and activating transcription factor 6 (ATF6) (Hetz, 2012; Wang and Kaufman, 2014; Wu and Kaufman, 2006).

Under normal physiological conditions, PERK, IRE1, and ATF6 proteins are maintained in an inactive state by binding to BiP/glucose-regulating protein 78 (GRP78), an important ER chaperone. Upon ER stress, BiP disassociates from these proteins and preferentially binds to the misfolded proteins in the ER lumen (Figure 1). Upon release from BiP, PERK is auto-phosphorylated leading to a cascade of signals including phosphorylation of eukaryotic translation initiation factor 2 α (eIF2 α) and translation of activating transcription factor-4 (ATF4), which induces transcription of C/EBP homologous protein (CHOP) (Harding et al., 1999; Hetz, 2012; Ma et al., 2002). The PERK/eIF2 α axis is also involved in the termination of the UPR after the stress has been relieved by activation of growth arrest and DNA damage-inducible protein, GADD34 (Ron and Walter, 2007). Another ER transmembrane sensor, IRE1, also becomes activated by autophosphorylation during ER stress. Through its endonuclease activity, IRE1 promotes splicing of a 26-base intron from X-box-binding protein 1 (XBP1) mRNA (Flamment et al., 2012). Spliced XBP1 (sXBP1) increases the number of ER chaperones and other components in order to boost the folding capacity of the ER (Tirasophon et al., 1998). Lastly, once activated, ATF6 moves from the ER to the Golgi apparatus to be cleaved by site-1 proteases (Haze et al., 1999). The cleaved N-terminal fragment of ATF6 is then transported to the nucleus where it acts in combination with sXBP1 to increase the levels of proteins that function to alleviate ER stress (Flamment et al., 2012). Activation of all three pathways alleviates ER stress by regulating gene expression and protein synthesis. The main purpose of the UPR is to increase the protein folding

capacity of the cell while decreasing the unfolded protein load (Harding et al., 1999; Hetz, 2012; Ma et al., 2002).

While chronic, unmitigated ER stress can lead to cell death, accumulating evidence suggests that a low level of ER stress may be protective to cells by eliciting an adaptive UPR that ‘preconditions’ the cell to a subsequent pathological insult, a process named ER hormesis. The UPR has been found to regulate autophagy, mitochondrial biogenesis and function, and the expression of various anti-oxidant molecules to protect mammalian cells under stressed conditions (Mollereau et al., 2014). Low levels of ER stress and the UPR also play an important role in the survival, self-renewal, and differentiation of stem cells in various organs (van Galen et al., 2014). Moreover, it is increasingly evident that different arms of the UPR may have a distinct role in regulation of cell fate in naïve and diseased states (Hetz, 2012; Wang and Kaufman, 2014; Wu and Kaufman, 2006).

In recent years, several articles have been published highlighting the potential role of ER stress in the regulation of skeletal muscle development, regeneration, and adaptation in response to external stimuli. In the following section, we provide a succinct review of the role and mechanisms of action of ER stress-induced UPR pathways in the regulation of various aspects of skeletal muscle function. We also provide potential avenues for future research that will elucidate the role of individual arms of the UPR in the regulation of skeletal muscle mass.

Myogenesis

Myogenesis is a complex and highly regulated process that involves the determination of multi-potential mesodermal cells to give rise to myoblasts, exit of these myoblasts from the cell cycle, and their differentiation into muscle fibers (Bentzinger et al., 2012). Myogenesis is regulated by the sequential expression of myogenic regulatory factors (MRFs), a group of basic helix-loop-helix transcription factors that include: Myf5, MyoD, myogenin, and MRF4 (Figure 2). Many steps of myogenesis can be recapitulated *in vitro* through incubation of primary myogenic cells or C2C12 myoblasts, which form multi-nucleated myotubes in low serum conditions (Bentzinger et al., 2012; Buckingham et al., 2003).

An essential feature of skeletal muscle formation is selective apoptosis that eliminates differentiation-incompetent myoblasts during myogenesis (Fidzianska and Goebel, 1991; Olson, 1992). Activation of ER stress initiates the activation of caspase-12, which leads to the downstream activation of caspase-9 and caspase-3 and subsequent apoptosis. An earlier study demonstrated that the ATF6 arm of the UPR and other markers of ER stress such as CHOP and BiP as well as caspase-12 are increased in the myoblasts undergoing apoptosis upon induction of myogenic differentiation (Nakanishi et al., 2005). Inhibition of ATF6 or caspase-12 reduced myoblast apoptosis and attenuated the formation of multi-nucleated myotubes in C2C12 cultures. Interestingly, a similar increase in caspase-12 is also observed in skeletal muscle during embryonic development suggesting that the ATF6 arm of the UPR leads to selective apoptosis of a subpopulation of cells that may be vulnerable to cellular stresses both *in vitro* and *in vivo* (Nakanishi et al., 2005). In support of these findings, a subsequent study demonstrated that forced activation of ER stress could be beneficial to improve myofiber formation (Nakanishi et al., 2007). Treatment of myoblasts with the ER

stressors tunicamycin or thapsigargin leads to increased apoptosis in C2C12 cultures after induction of differentiation. Interestingly, the remaining myoblasts were resistant to apoptosis and were able to efficiently differentiate into healthy and functional myotubes that were morphologically distinct from the untreated cells, further suggesting that ER stress plays an important role in selectively removing differentiation incompetent myoblasts during myogenesis (Nakanishi et al., 2007).

While the ATF6 arm of the UPR appears to promote selective apoptosis of myoblasts, other arms of the UPR also appear to have a role in the regulation of myogenesis. The PERK/eIF2 α arm of the UPR and its downstream target, CHOP, are transiently activated in a subset of myoblasts after initiation of the differentiation program (Alter and Bengal, 2011). Increased expression of CHOP inhibits myogenic differentiation through repressing the transcription of MyoD, which could be a mechanism to prevent premature differentiation of myoblasts during myogenesis. Interestingly, overexpression of a phosphorylation resistant mutant of eIF2 α (i.e. eIF2 α S51A) also causes increased cell death during myogenesis even though the mechanisms remain unknown (Alter and Bengal, 2011). ER stress transducer IRE1 is an endonuclease, which removes an intron from XBP1 resulting in synthesis of the potent transcription factor, sXBP1 (Wang and Kaufman, 2014). Previously, it has been reported that *Xbp1* is a direct target gene of MyoD and myogenin transcription factors, thus, providing an important link between XBP1 and myogenesis (Blais et al., 2005). Recently, XBP1 has been found to regulate the expression of a large subset of genes in both myoblasts and myotubes including those involved in maintenance of ER function, growth, and DNA damage and repair pathways (Acosta-Alvear et al., 2007). Overexpression of sXBP1 inhibits the expression of several markers of myogenesis and leads to formation of smaller myotubes in C2C12 cultures after their incubation in differentiation medium. Interestingly, XBP1 directly activates the expression of the Mist1 transcription factor, a critical negative regulator of MyoD, which is essential for myogenesis. Although genetic evidence about the role of XBP1 in skeletal muscle formation is still missing, these cell culture studies suggest that XBP1 regulates differentiation of myoblasts into multinucleated myotubes (Acosta-Alvear et al., 2007).

Myogenesis is also required for the regeneration of skeletal muscle upon injury. Satellite cells are the adult muscle stem cells that contribute to skeletal muscle repair in adults. In normal conditions, these cells reside between the sarcolemma and the basal lamina in a quiescent state. Upon muscle injury, satellite cells get activated, proliferate and give rise to myoblasts/myocytes which eventually fuse with injured myofibers (Relaix and Zammit, 2012; Yin et al., 2013). While the majority of satellite cells differentiate into the myogenic lineage, a fraction of them undergo self-renewal and return to a quiescent state. The paired box 7 (Pax7) transcription factor is essential for self-renewal and maintenance of the myogenic potential of satellite cells (Bentzinger et al., 2012; Buckingham et al., 2003). Zismanov et al recently reported that PERK and its downstream target eIF2 α are constitutively phosphorylated in quiescent satellite cells and this phosphorylation is reduced upon activation of the satellite cells (Zismanov et al., 2016). Interestingly, targeted inducible expression of a phosphorylation resistant mutant of eIF2 α (i.e. eIF2 α S51A) or deletion of PERK leads to loss of quiescence in satellite cells, characterized by the increased expression of Myf5 and MyoD. While unable to undergo self-renewal, satellite cells expressing the

eIF2 α S51A mutant are capable of undergoing terminal differentiation and subsequent fusion with myofibers. The study also showed that inhibition of eIF2 α dephosphorylation using small molecule such as Sal003 promotes satellite cell self-renewal *ex vivo*. Remarkably, satellite cells treated with Sal003 retain their myogenic capacity when transplanted in skeletal muscle *in vivo* (Zismanov et al., 2016). However, the results of this study are somewhat contradictory to a previously published study where it was found that overexpression of eIF2 α S51A causes cell death and diminishes the differentiation of cultured myoblasts (Alter and Bengal, 2011). This could be attributed to the different experimental approaches and myogenic cell types utilized in these two studies (Alter and Bengal, 2011; Zismanov et al., 2016). However, it remains unknown whether the inhibition of PERK dephosphorylation will also promote satellite cell self-renewal and maintenance of their differentiation potential when transplanted into injured skeletal muscle. It is notable that in addition to PERK, eIF2 α can also be phosphorylated by other kinases such as double stranded RNA-activated protein kinase R, heme-regulated inhibitor eIF2 α kinase, and general control nonderepressible-2 (GCN2) in different conditions (Kilberg et al., 2009). Furthermore, PERK also has several eIF2 α -independent functions, which are collectively known to promote cell survival in stress conditions (Avivar-Valderas et al., 2011; Cavener et al., 2010; Lu et al., 2004; Mollereau et al., 2014). Finally, the role of the IRE1/XBP1 and the ATF6 arms of the UPR have yet to be investigated through molecular and genetic approaches in the regulation of satellite cell self-renewal, proliferation, and differentiation. Nevertheless, these published results suggest that the UPR plays an important role in the regulation of myogenesis both *in vitro* and *in vivo*.

Skeletal Muscle Atrophy

Skeletal muscle atrophy is a devastating complication that contributes to both morbidity and mortality (Bonaldo and Sandri, 2013). Skeletal muscle mass is maintained through a delicate balance between the rate of protein synthesis and the rate of protein degradation. The ubiquitin-proteasome system (UPS) is a major proteolytic mechanism in skeletal muscle (Cohen et al., 2009; Du et al., 2004). Several E3 ubiquitin ligases such as: MuRF1, MAFBx (Atrogin-1), Nedd4.1, TRAF6, and MUSA1, have now been identified which mediate proteolytic degradation of both thick and thin filament proteins in skeletal muscle (Bodine and Baehr, 2014; Bonaldo and Sandri, 2013). The autophagy-lysosomal system (ALS) is another proteolytic mechanism involved in the degradation of defunct organelles and misfolded proteins (Sandri, 2010). While inhibition of basal autophagy can lead to myopathies due to the accumulation of dysfunctional organelles and oxidative stress (Carnio et al., 2014; Masiero et al., 2009), excessive activation of autophagy can lead to muscle wasting (Bonaldo and Sandri, 2013; Penna et al., 2013; Sandri, 2008; Sandri et al., 2004).

The activity of the UPS and ALS are regulated through the activation of a number of signaling pathways such as p38 MAPK, AMP-activated protein kinase (AMPK), and nuclear factor-kappa B (NF- κ B), which function through modulating the activity of various transcriptional regulators (Bonaldo and Sandri, 2013; Li et al., 2008). The IGF-1/PI3K/Akt/mTOR cascade is a major signaling pathway that increases the rate of protein synthesis in skeletal muscle (Egerman and Glass, 2014). In addition, activation of this pathway also inhibits protein degradation in skeletal muscle (Bonaldo and Sandri, 2013; Egerman and

Glass, 2014). Accumulating evidence also suggests that changes in mitochondrial content, integrity, and function also play a critical role in the regulation of skeletal muscle mass (Bonaldo and Sandri, 2013; Romanello et al., 2010).

Activation of ER stress and the UPR occurs in skeletal muscle due to exercise, aging, and many other perturbations (Figure 1). However, their role in the regulation of skeletal muscle mass and function remain poorly understood. Moreover, in most of the conditions, all three arms of the UPR are activated, making it difficult to ascertain the contribution of each pathway to skeletal muscle adaptation. To understand the ER stress independent role of the PERK arm of the UPR in skeletal muscle, Miyake et al recently generated Fv2E-PERK transgenic (Tg) mice. In this mouse line, a fusion protein of artificial dimerization domain (Fv2E) and PERK kinase domain were overexpressed in skeletal muscle (Miyake et al., 2016). The Fv2E-PERK Tg mouse serves as a model for ligand-dependent PERK activation. Interestingly, adult Fv2E-PERK Tg mice showed reduced body weight and a reduction in individual hind limb muscle weight as well. Accordingly, grip strength was also significantly reduced in Fv2E-PERK Tg mice compared to littermate control mice. While Fv2E-PERK Tg mice show signs of muscle atrophy, constitutive activation of PERK in skeletal muscle leads to increased expression of metabolic and antioxidant genes. Moreover, PERK stimulates the production of fibroblast growth factor 21 (FGF21), which induces energy expenditure in brown adipose tissue and prevents adiposity (Miyake et al., 2016). These findings suggest that PERK plays a dual role in skeletal muscle. While it reduces muscle mass potentially due to inhibition of translation, it also improve muscle health through augmenting anti-oxidant mechanisms. Although floxed PERK mice have been generated, the effect of deletion of PERK in skeletal muscle in naïve as well as in catabolic conditions has not been investigated yet. Similarly, the role of IRE1 and ATF6 α in skeletal muscle homeostasis using genetic mouse models has yet to be investigated. Based on their divergent downstream targets, it would not be surprising that individual arms of the UPR play distinct roles in skeletal muscle in various physiological and pathological conditions.

The activation of ER stress markers and the UPR in skeletal muscle has been studied in multiple atrophy conditions. An earlier study reported no significant changes in the expression of ER stress proteins such as GRP78, calreticulin, CHOP, vinculin, the type I D-myo-inositol 1,4,5-trisphosphate receptor, protein kinase R, and eIF2 α in skeletal muscle in response to hind limb unloading, a model of disuse atrophy (Hunter et al., 2001). However, BiP and CHOP are shown to be increased 14 days after reloading the muscle. This effect was more pronounced in the older mice (Baehr et al., 2016). Furthermore, several markers of ER stress including CHOP are increased in skeletal muscle in response to denervation and in AR113Q mice, an established model of spinal and bulbar muscular atrophy (SBMA) (Yu et al., 2011). Interestingly, deletion of CHOP accentuated muscle atrophy in normal mice after denervation and in the AR113Q mice. Although, there wasn't a noticeable difference in the activity of the UPS, autophagy was significantly increased upon deletion of CHOP in the skeletal muscle of both these models. In contrast, inhibition of autophagy through Beclin-1 haplosufficiency attenuated muscle atrophy in response to denervation in normal mice and increased the lifespan of AR113Q mice (Yu et al., 2011). Although the mechanisms by which CHOP inhibits autophagy remain unknown, these findings suggest that downstream of ER stress, induction of CHOP is a mechanism to prevent excessive loss of muscle mass in

response to denervation and during SBMA. ER stress and the UPR pathways are also activated in SOD1 models of ALS (Chen et al., 2015; Hetz et al., 2009). Interestingly, it was found that induction of autophagy following XBP1 deletion ameliorated pathology of SOD1 mice. In this model, autophagy promoted the degradation of mutant SOD1 resulting in the clearance of aggregates (Hetz et al., 2009). These reports emphasize that the activation of specific UPR pathways may have differential outcomes on skeletal muscle mass depending on the underlying condition and muscle wasting stimuli.

ER stress has also been found to be elevated in the skeletal muscle of the elderly, which could be due to reduced expression of ER chaperones (Chalil et al., 2015; Deldicque, 2013; O'Leary et al., 2013; Ogata et al., 2009). Anabolic resistance is considered one of the important mechanisms in age-associated muscle atrophy. However, a recent study has shown that ER stress may not contribute to age-associated anabolic resistance in the skeletal muscle of mice (Chalil et al., 2015). Additionally, activation of ER stress has been implicated in diaphragm contractile dysfunction in a mouse model of sepsis (Jiao et al., 2017).

As suggested, one of the important consequences of ER stress is the induction of autophagy in skeletal muscle. However, the signaling link between the UPR and autophagy remains less understood. Protein Kinase C theta (PKC θ) appears to be essential for the induction of autophagy in response to ER stress (Madaro et al., 2013). ER stressor thapsigargin or tunicamycin strongly activates PKC θ in both cultured myoblasts and myotubes. PKC θ activation was localized into LC3-positive autophagosomes in thapsigargin-treated myotubes. The role of PKC θ in ER stress-induced autophagy was evident by the findings that inhibition PKC θ *in vitro* prevented ER stress induced activation of autophagy, but had no effect on activation of ER stress (Madaro et al., 2013). *In vivo*, lack of PKC θ prevented the activation of autophagy and skeletal muscle atrophy in response to starvation or immobilization. These findings suggest that PKC θ functions as an ER stress sensor and is required for ER stress-dependent activation of autophagy in skeletal muscle (Madaro et al., 2013).

TRAF6 is an E3 ubiquitin ligase, and through association with the dimeric ubiquitin-conjugating enzyme Ubc13/Uev1A, it catalyzes lysine 63 (K63)-linked polyubiquitination of several target proteins. TRAF6 functions as a central regulator in multiple signaling pathways, such as NF- κ B, MAPK, and PI3K/Akt, in response to certain cytokines and microbial products. Targeted deletion of TRAF6 prevents skeletal muscle wasting in distinct catabolic conditions including starvation, denervation, and cancer cachexia (Paul et al., 2012; Paul et al., 2010). TRAF6 appears to be one of the upstream regulators for the induction of the UPR in skeletal muscle, especially in response to starvation. Our studies have shown that deletion of TRAF6 reduces the levels of multiple markers of ER stress such as ATF4, CHOP, GRP94, and GADD34 in the skeletal muscle of fasting mice (Paul et al., 2012). However, the molecular interactions through which TRAF6 induces ER stress and UPR pathways in skeletal muscle remain to be investigated.

ER stress also appears to play an important role in the regulation of skeletal muscle mass during cancer cachexia. We recently reported that several markers of ER stress are highly up-regulated in the skeletal muscle of lewis lung carcinoma (LLC)-bearing mice and

APC/Min+ mice, the two established models of cancer cachexia (Bohnert et al., 2016). We also found that conditioned media from LLC cells was sufficient to induce the phosphorylation of eIF2 α , splicing of XBP1, and gene expression of several downstream ER stress markers suggesting that tumor-derived factors are responsible for the activation of ER stress in skeletal muscle. Interestingly, pan-inhibition of ER stress using 4-PBA a chemical chaperone known to attenuate ER stress both *in vivo* and *in vitro* (Ozcan et al., 2006; Zode et al., 2015), led to accelerated muscle loss in LLC-bearing mice (Bohnert et al., 2016). More importantly, chronic administration of 4-PBA caused significant muscle atrophy even in naïve mice. Furthermore, 4-PBA induced rapid atrophy in cultured myotubes, which was further accentuated in the presence of LLC conditioned medium. Inhibition of the UPR reduced the activity of the Akt/mTOR pathway and increased the expression of components of the UPS and the ALS in LLC-bearing mice (Bohnert et al., 2016). ATF4 is one of the important transcription factors that is also induced through the activation of the PERK arm of the UPR. Published results suggest that ATF4 mediates muscle wasting during starvation (Paul et al., 2012) through activation of growth arrest and DNA damage-inducible 45a protein (Ebert et al., 2012). While we found increased expression of ATF4 in the skeletal muscle of LLC-bearing mice, this increase was completely abolished by 4-PBA. However, we still observed accelerated muscle atrophy. These findings suggest that ATF4 may not be involved in muscle wasting during cancer cachexia or since 4-PBA is a pan-ER stress inhibitor, it also may have inhibited other components of ER stress that have a role in the preservation of muscle mass. Although 4-PBA is one of the most commonly used pharmacological inhibitors of ER stress, a caveat of this study was that the prolonged use of 4-PBA can also influence some metabolic pathways in addition to its effect on the ER stress pathway (Bohnert et al., 2016). Therefore, the exact role and contribution of the individual arms of the UPR in regulation of skeletal muscle mass needs to be further investigated using genetic mouse models. Nevertheless, the study provided initial evidence that the activity of the UPR pathways are essential for maintaining muscle mass in naïve conditions and during cancer cachexia. Taken together, it is now evident that ER stress and the UPR pathways are activated in numerous conditions that lead to skeletal muscle atrophy and depending on the stimulus, it can have both positive and negative effects in the regulation of skeletal muscle mass.

Skeletal Muscle Adaptation to Exercise

Exercise training has been shown to preserve the aerobic fitness of skeletal muscle as well as skeletal muscle strength. These adaptations can ameliorate metabolic dysfunction and prevent chronic disease. Aerobic and resistance exercise pose different challenges to skeletal muscle and elicit different molecular mechanisms in skeletal muscle as well. Published reports suggest that exercise activates all three ER stress responsive UPR pathways (Miyake et al., 2016; Wu et al., 2011). ER stress markers such as BiP, GRP94, GADD34, ATF4, CHOP, and sXBP1 are induced after one bout of exhaustive treadmill running (Wu et al., 2011). An additional study showed that ER stress markers p-IRE1, p-PERK, and p-eIF2 α are increased in the skeletal muscle of mice trained on a downhill treadmill (Pereira et al., 2016). However, when the mice had received previous training in the exercise paradigm, only the ER chaperones BiP and GRP94 were found to be increased in skeletal muscle, suggesting that the activation of the UPR is a mechanism for skeletal muscle adaptation after

initial exercise training (Wu et al., 2011). A similar rapid increase in the markers of the UPR was observed when rats were subjected to chronic contractile activity and this increase was attenuated with repeated bouts (Memme et al., 2016). Furthermore, increases in ER stress markers such as BiP and sXBP1 have also been characterized in human muscle biopsies after a bout of running (Kim et al., 2011).

The transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 α) is a key regulator of mitochondrial biogenesis and metabolic function (Handschin et al., 2007; Lin et al., 2002). Levels of PGC-1 α are induced in skeletal muscle following an acute exercise bout or prolonged exercise training (Baar et al., 2002; Russell et al., 2003). Moreover, overexpression of PGC-1 α in the skeletal muscle has been found to improve performance capacity during voluntary and forced exercise in mice (Calvo et al., 2008). Interestingly, PGC-1 α is necessary for the activation of the UPR during pharmacologically induced ER stress and exercise training (Wu et al., 2011). PGC-1 α interacts with cleaved ATF6 α to induce an adaptive UPR in skeletal muscle after exercise (Wu et al., 2011). The role of ATF6 α in exercise-induced adaptive response is also evidenced by the findings that ATF6 α mice fail to efficiently recover from muscle damage that occurs after acute exercise. While the ATF6 α arm of the UPR promotes skeletal muscle adaptation in response to exercise, some parts of the UPR may also have detrimental effects on skeletal muscle adaptation and recovery following exercise training. This is supported by the findings that genetic deletion of CHOP improved exercise tolerance in skeletal muscle specific PGC-1 α knockout mice (Wu et al., 2011). Although more studies are needed, it is plausible that the PERK-eIF2 α arm of the UPR which also leads to the induction of CHOP, negatively regulate skeletal muscle homeostasis following exercise training.

During resistance exercise, skeletal muscle is subjected to a short bout of maximal contraction. These exercises cause the skeletal muscle to adapt through augmenting protein synthesis and organelle biogenesis. Synergistic ablation is a model used to study skeletal muscle hypertrophy, similar to that seen from resistance exercise. In this model, the gastrocnemius and soleus muscles are partially ablated leaving the plantaris muscle as the lone hind limb extensor. Hamilton et al. hypothesized that a high rate of protein synthesis, which occurs during muscle hypertrophy, results in ER stress and activation of the maladaptive UPR. This is characterized by an increase in CHOP and a decrease in IRE1 α at 12 days post-ablation, possibly leading to a decrease in protein synthesis in an attempt to halt the excessive muscle growth (Hamilton et al., 2014). ER stress has also been examined in humans following resistance exercise where participants completed a unilateral single bout of resistance exercise using the knee extensors. The biopsies from these muscles indicated an increase in the protein levels of BiP, PERK, and IRE1 48h post-exercise. However, the mRNA levels of IRE1 as well as ATF6 were unchanged (Ogborn et al., 2014).

Altogether, these studies indicate that moderate exercise initially causes induction of ER stress and activation of UPR pathways. However, after multiple rounds of training, these markers appear to be diminished, which suggests that the UPR becomes activated to novel or challenging exercises in skeletal muscle during both endurance and resistance exercises. The activation of ER stress leads to some mode of adaption in skeletal muscle that allows the

skeletal muscle to acclimate to the demands of a specific exercise. More investigations are needed to understand the molecular mechanisms by which ER stress-induced UPR pathways promote skeletal muscle adaptation following exercise.

Skeletal Muscle Metabolism

Obesity and type II diabetes are growing concerns to the healthcare and research community. The common characteristic between these diseases is a decrease in insulin action on target tissues, defined as insulin resistance (IR) (Samuel and Shulman, 2012). Activation of ER stress has been associated with development of IR (Ozcan et al., 2004) and inhibition of ER stress via the chemical chaperone 4-PBA was demonstrated to prevent development of IR in obese and diabetic mice (Ozcan et al., 2006). Skeletal muscle is one of the major sites of glucose disposal in the body. Recent studies have indicated that ER stress plays a role in induction of IR, specifically in skeletal muscle. *In vivo*, high-fat diets have been shown to lead to induction of ER stress in skeletal muscle (Deldicque et al., 2010; Rieusset et al., 2012).

Tribbles 3 (TRB3) is a pseudokinase that is expressed in multiple tissues including: liver, adipose tissue, heart, and skeletal muscle (Eyers et al., 2016). A seminal study has previously shown that TRB3 disrupts insulin signaling in the liver by binding directly to Akt and blocking activation of the kinase (Du et al., 2003). Overexpression of TRB3 in C2C12 myotubes also prevented insulin-stimulated glucose uptake through inhibiting phosphorylation of Akt (Koh et al., 2006). Additionally, TRB3 is increased in the skeletal muscle of patients with type II diabetes (Liu et al., 2010). Interestingly, activation of ER stress via tunicamycin and thapsigargin leads to increases in TRB3 levels in skeletal muscle. Moreover, knockdown of TRB3 in mice receiving a high-fat diet prevented the impairment in insulin receptor substrate (IRS) Tyr⁶¹² signaling and restored glucose uptake in skeletal muscle (Koh et al., 2013).

AMPK is also known to affect metabolism through enhancement of glucose utilization by increasing the expression of glucose transport protein 4 (GLUT4). When activated, GLUT4 is translocated to the plasma membrane, where it increases glucose uptake in skeletal muscle, independent of insulin (Hayashi et al., 2000). Furthermore, mice lacking AMPK2 α display a reduction in glucose utilization in skeletal muscle (Viollet et al., 2003). Interestingly, treatment of L6 myotubes with ER stressors caused a reduction in AMPK activity. The reduction in AMPK activity was attributed to an increase in extracellular signal-regulated kinase (ERK) phosphorylation. Treatment of the myotubes with a pharmacological inhibitor of ERK or silencing of ERK using siRNA restored AMPK phosphorylation and glucose uptake following the induction of ER stress (Hwang et al., 2013).

The role of AMPK in skeletal muscle regulation of metabolism was further demonstrated in a study examining the effect of Bortezomib on C2C12 myotubes stimulated with palmitic acid. Bortezomib is an anti-cancer agent that induces ER stress by inhibiting proteasomal degradation under normal conditions. Interestingly, co-treatment of myotubes with both Bortezomib and palmitic acid prevented activation of ER stress. Furthermore, co-treatment prevented a reduction in phosphorylation of Akt and glucose uptake and caused an increase

in AMPK phosphorylation. Moreover, the protective effects of Bortezomib were diminished after knockdown of AMPK (Kwak et al., 2016). These studies indicate a role for ER stress in inhibition of AMPK activity and subsequent reduction in glucose uptake.

Obesity and type II diabetes are often also associated with low-grade inflammation (Greenberg and Obin, 2006). Mice receiving a high-fat diet have been shown to have increases in toll-like receptors (TLRs) and inflammatory cytokines. This increase in inflammation occurs partially through ER stress mechanisms. This is demonstrated in a study showing that mice lacking TLR4 have reduced ER stress following a high-fat diet (Pierre et al., 2013). Peroxisome proliferator-activated receptors (PPARs) are a nuclear receptor superfamily of transcription factors that have been shown to reduce inflammation (Daynes and Jones, 2002). Activation of PPAR β/γ by agonist GW501516 prevented ER induction by palmitate, thapsigargin and tunicamycin in C2C12 myotubes. Furthermore, the activation of PPAR β/γ prevented ER stress associated inflammation and insulin resistance. Myotubes treated with compound C, an AMPK inhibitor, failed to demonstrate the beneficial effect of PPAR β/γ , indicating an essential role of AMPK in PPAR β/γ prevention of inflammation and insulin resistance (Salvado et al., 2014). Activation of PPAR α through the agonist fenofibrate has also been shown to improve ER stress associated inflammation and insulin resistance in skeletal muscle. Mice on a high-fat diet that received fenofibrate showed a reduced body weight and improved insulin sensitivity. In the skeletal muscle specifically, mice treated with fenofibrate showed diminished ER stress and a reduction in inflammation. Lastly, C2C12 cells treated with fenofibrate had improved glucose uptake following stimulation with palmitic acid (Dai et al., 2016).

Myokines, the cytokines secreted by skeletal muscle, have been found to have a positive effect on whole body metabolism. Specifically, fibroblast growth factor 21 (FGF21) secreted from skeletal muscle protects the body from obesity and IR (Kim et al., 2013). In contrast, mice lacking FGF21 become obese and glucose intolerant (Badman et al., 2009). Interestingly, mice that lack tuberous sclerosis complex 1 (TSC1) results in a constitutively active mammalian target of rapamycin complex 1 (mTORC1) and they have an increase in plasma concentrations of FGF21. Moreover, these mice have increased insulin sensitivity and fatty acid oxidation. Furthermore, activation of mTORC1 triggers the activation of ER stress, specifically the PERK-eIF2 α -ATF4 arm of the UPR, which leads to the secretion of FGF21 (Guridi et al., 2015). As discussed above, the Fv2E-PERK Tg mice show increased expression of metabolic and antioxidant genes through production of FGF21. Secretion of FGF21 from the skeletal muscle suppressed fat accumulation by stimulating energy consumption in brown adipose tissue, but not in skeletal muscle (Miyake et al., 2016). These studies indicate a role of the PERK arm of the UPR in secretion of FGF21, and stimulation of this arm or endogenous FGF21 could be a useful therapy in counteracting type II diabetes.

These studies together indicate a role for ER stress and UPR pathways in skeletal muscle in the induction of insulin resistance. However, further investigation using knock-in and knock-down approaches are needed to understand the role of specific components of the UPR on skeletal muscle metabolic function.

Skeletal Muscle Diseases

Myopathies are muscle diseases in which myofibers are damaged as a result of inflammatory immune response (Lundberg, 2001). Specifically, myositis diseases involve inflammation in skeletal muscle leading to weakness, swelling, and pain. However, several studies have indicated that inflammation alone does not cause myositis (DeVere and Bradley, 1975), suggesting that the immune system is not acting alone. A conditional transgenic mouse in which class I major histocompatibility complex (MHC-I) is overexpressed in skeletal muscle is commonly used as a model for myositis. Interestingly, ER chaperone protein BiP is increased in the skeletal muscle of these transgenic mice (Nagaraju et al., 2005). In a separate study, mice overexpressing MHC-I were crossed with the immunodeficient Rag2^{-/-} mouse to examine if the upregulation of ER stress is due to the inflammatory response. Interestingly, prevention of the immune response still caused myositis to occur as well as an increase in the ER stress markers BiP and ATF6 (Freret et al., 2013).

Sporadic inclusion-body myositis (s-IBM) is the most common degenerative muscle disease in adults over the age of 50. The hallmark of this disease is vacuolar muscle fibers, intramuscular fiber inclusions, and various degrees of mononuclear cell inflammation (Askanas and Engel, 2001; Askanas and Engel, 2002). An intriguing feature of this disease is that like Alzheimer's disease, it is characterized by accumulation of amyloid- β (A β), and phosphorylated tau proteins (Askanas and Engel, 2001; Askanas and Engel, 2002). Interestingly, muscle biopsies from patients with s-IBM indicate that all major ER chaperones are increased and associate with the A β proteins in skeletal muscle (Vattemi et al., 2004). This indicates a possible role for ER chaperone proteins in the proper folding and trafficking of A β in skeletal muscle. In a separate study, muscle biopsies of s-IBM patients indicated an increase in other ER stress markers including ATF4, CHOP, ATF6, and XBP1 in skeletal muscle (Nogalska et al., 2015).

Myasthenia gravis (MG) is an autoimmune disorder that affects the neuromuscular junction targeted by pathogenic autoantibodies to acetylcholine receptors (AChR). These autoantibodies impair the postsynaptic membrane by inducing complement-mediated damage, blocking the interaction of acetylcholine to the AChR causing degradation of AChR (Meriggioli and Sanders, 2009). This results in patients with MG experiencing weakness and fatigability. Interestingly, it has been shown that activation of ER stress in C2C12 myotubes by tunicamycin causes increased degradation of the AChRs by endocytosis. Furthermore, silencing of XBP1, a downstream target of IRE1, using siRNA in C2C12 myotubes slowed the degradation of AChR by endocytosis (Du et al., 2016). Moreover, skeletal muscle obtained from these patients exhibited an increase in BiP and GRP94 (Iwasa et al., 2014; Suzuki et al., 2011).

Duchenne muscular dystrophy (DMD) is the most common muscular dystrophy and is characterized by a loss of the dystrophin protein leading to inflammation, cell infiltration, fibrosis, and necrosis (Tidball and Wehling-Henricks). The mdx mouse model is commonly used as a model for DMD. Analysis of skeletal muscle from multiple studies in dystrophic mice showed an increase in numerous ER stress markers including GRP78, PERK, eIF2 α , IRE1, and sXBP1 (Hulmi et al., 2016; Moorwood and Barton, 2014). Myotonic dystrophy 1 (DM1) is an inherited muscular dystrophy caused by an abnormal amount of CTG

trinucleotide repeats in the 3' untranslated region of the dystrophin protein kinase (DMPK) gene. Analysis of skeletal muscle biopsies from patients with DM1 showed increases in ER stress markers such as GRP78, PERK, eIF2 α , and XBP1 (Ikezoe et al., 2007). Tibial muscular dystrophy (TMD) is an autosomal dominant myopathy caused by heterogeneous mutation in the last two exons of the *Titin* gene and is characterized by atrophy and weakness in the muscles of the anterior compartment of the lower leg (Udd, 1992). Gene profiling studies on muscle biopsies from TMD patients suggested that one of the potential pathophysiological mechanisms was ER stress, evidenced by increased levels of sXBP1 (Screen et al., 2014). It is notable that while the markers of ER stress have been found to be elevated in various muscle disorders, their role in disease progression has not been yet elucidated using pharmacological or genetic approaches.

Concluding Remarks

From the discussion above, it is clear that ER stress and the UPR are activated in skeletal muscle in multiple conditions. However, because of the complexity of multiple UPR pathways and several downstream targets, their exact role remains poorly understood. It is now increasingly evident that depending on the underlying condition, activation of ER stress-induced UPR may produce beneficial or deleterious effects. It is also clear that the UPR plays an important physiological role in skeletal muscle adaptation in response to acute exercise. Furthermore, low levels of ER stress may be beneficial in maintaining the pool of satellite cells in adult skeletal muscle for regenerative myogenesis. While there are several studies suggesting that ER stress and the UPR pathways are perturbed in skeletal muscle in conditions of atrophy and hypertrophy, it remains unknown how these pathways regulate skeletal muscle mass. Consistent with the notion that chronic unresolved stress can lead to insulin resistance, inflammation, and cell death, the markers of ER stress have been found to be elevated in skeletal muscle in models of metabolic disorders and in a number of muscle degenerative diseases. A few studies have also shown that reducing ER stress improves insulin sensitivity and glucose uptake in skeletal muscle. However, it remains unknown whether the inhibition of ER stress will improve myopathy in various muscle degenerative disorders. Altogether, available literature suggests that the modulation of ER stress and UPR pathways can have therapeutic importance for skeletal muscle in diverse conditions and disease states. Interestingly, a number of ER stress activators and inhibitors have now been developed and can potentially be used for skeletal muscle diseases as well. However, before using pharmacological modulators of ER stress, more specific perturbation using molecular and genetic approaches are needed to tease out the mechanisms by which the components of ER stress and UPR pathways alter skeletal muscle homeostasis.

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Figure 1. Schematic representation of the unfolded protein response pathways in skeletal muscle Under normal physiological conditions, the PERK, IRE1, and ATF6 proteins are maintained in an inactive state by binding to BiP. Stress in skeletal muscle caused by aging, disease, exercise, starvation, cachexia, denervation, or high fat diet causes BiP to disassociate from these proteins and preferentially binds to the misfolded proteins in the ER lumen. Upon release from BiP, PERK is auto-phosphorylated leading to a cascade of signals including phosphorylation of eIF2 α and translation of ATF4, a potent transcription factor. Another ER transmembrane sensor, IRE1, also becomes activated by autophosphorylation during ER stress. Through its endonuclease activity, IRE1 promotes splicing of a 26-base intron from XBP1 mRNA as well as phosphorylation of JNK. Lastly, once activated, ATF6 moves from the ER to the Golgi apparatus to be cleaved by site-1 proteases. The cleaved N-terminal fragment of ATF6 is then transported to the nucleus where it acts in combination with sXBP1 and ATF4 to alleviate ER stress by regulating gene expression and protein synthesis. In addition to regulation of ER related genes, activation of ER stress has been shown to increase skeletal muscle atrophy by inhibition of protein synthesis through eIF2 α , activation of JNK through IRE1, and potentially through augmenting the expression of components of UPS and ALS. Activation of ATF6 increases skeletal muscle adaptation by increasing expression of metabolic genes through interaction with PGC1 α . ER stress has also been linked to insulin resistance through activation of ERK signaling and inhibition of AMPK. However, specific activation of PERK signaling leads to secretion of FGF21, which is shown to alleviate insulin resistance.

Figure 2. Schematic diagram illustrating the effect of ER stress pathways on myogenesis
 Adult muscle stem cells or satellite cells reside between the sarcolemma and the basal lamina of mature muscle in a quiescent state and are maintained by constitutively phosphorylated PERK and phosphorylated eIF2α. Dephosphorylation of PERK and eIF2α promotes progression and commitment of satellite cells to the myogenic lineage. Prior to differentiation, myoblasts undergo an apoptotic pruning process facilitated by ATF6, ensuring that only differentiation-competent myoblasts proceed. Competent myoblasts differentiate into myocytes; however, CHOP has been found to inhibit this transition. Myocytes fuse with one another to form multi-nucleated myotubes. IRE1 and sXBP1 have been shown to disrupt the fusion process, resulting in thin, unhealthy cells through inhibition of MyoD and Myogenin. Myotubes undergo a maturation process to form adult myofibers with their own supply of quiescent satellite cells available for repair of damaged tissue due to injury or disease.