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Mitochondrial and Skeletal Muscle Health with Advancing Age

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Abstract

With increasing age there is a temporal relationship between the decline of mitochondrial and skeletal muscle volume, quality and function (i.e., health). Reduced mitochondrial mRNA expression, protein abundance, and protein synthesis rates appear to promote the decline of mitochondrial protein quality and function. Decreased mitochondrial function is suspected to impede energy demanding processes such as skeletal muscle protein turnover, which is critical for maintaining protein quality and thus skeletal muscle health with advancing age. The focus of this review was to discuss promising human physiological systems underpinning the decline of mitochondrial and skeletal muscle health with advancing age while highlighting therapeutic strategies such as aerobic exercise and caloric restriction for combating age-related functional impairments.

1.0 Introduction

Reports of skeletal muscle atrophy that accompany advancing age (i.e., sarcopenia) and the associated reductions in skeletal muscle function and quality have been observed for several decades (Critchley 1931, Rosenberg 1989, Rosenberg 1997). Recently, panels of leading scientists and physicians associated with large-scale epidemiological studies have created specific, objective criteria based on lean tissue mass and functional capacity to improve the diagnosis and treatment of sarcopenia (Delmonico et al. 2007, Fielding et al. 2011, Goodpaster et al. 2006, Morley et al. 2011, Newman et al. 2003). Human aging starts after the third decade and the progression of skeletal muscle atrophy with age is a slow process (~1% per year), but accelerates as humans approach 80 years of age (Baumgartner et al. 1998). With expansion in human lifespan, the elevated rate of muscle loss becomes more problematic since skeletal muscle is critical for functionality and substrate metabolism. When the substrate reservoir deteriorates with age, the associated cardiometabolic disease states (i.e. insulin resistance, diabetes, cardiovascular disease, obesity) become more prevalent (Atlantis et al. 2009). Many studies have observed reduced skeletal muscle mass and infiltration of adipose tissue depots within or between skeletal muscle groups that are associated with reduced muscle function, insulin resistance and obesity (Delmonico et al. 2009, Goodpaster et al. 2005, Goodpaster et al. 2000). A key link between a reduction in skeletal muscle health and prevalence of metabolic disorders with advancing age may be related to impaired mitochondrial function. A reduction in mitochondrial abundance and function with age has been observed across various species (*C. elegans*, *Drosophila*, mice, humans) and tissues (skin, nerve, brain, skeletal muscle). Moreover, perturbations in skeletal

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muscle mitochondrial energetics have been correlated with reduced aerobic capacity (Short et al. 2005a), walking capacity (Coen et al. 2012) and skeletal muscle function (Safdar et al. 2010) in older adults. The mechanisms of age-related changes in skeletal muscle are multifactorial but the purpose of this review is to highlight the apparent temporal and functional connection between the decline of mitochondrial and skeletal muscle health (Figure 1).

2.0 Reduced mitochondrial content and function with age

Electron microscopic assessment of skeletal muscle biopsy samples revealed lower mitochondrial volume density in older adults (Conley et al. 2000). A decline in mitochondrial content, as represented by mitochondrial DNA copy number, has also been demonstrated in rodents (Barazzoni et al. 2000) and humans (Short et al. 2005a). These findings, coupled with investigations that observed reduced levels of mitochondrial protein synthesis (Rooyackers et al. 1996) and expression of proteins encoded by both mitochondrial and nuclear DNA (Lanza et al. 2008, Short et al. 2005a), are expected to alter mitochondrial function. Semi-quantitative analyses, such as immunoblotting or maximal enzyme activity, support the notion that aging skeletal muscle contains less abundance of enzymes in oxidative metabolism (i.e. Krebs Cycle, beta-oxidation) and/or proteins involved in the electron transport chain (ETC) (Cooper et al. 1992, Ghosh et al. 2011, Lanza et al. 2008, Rooyackers et al. 1996, Tonkonogi et al. 2003, Trounce et al. 1989). Collectively, reductions in mitochondrial proteins and volume may limit ATP production for energy demanding processes such as myocellular remodeling to maintain protein quality.

Advancements of *in vitro* and *ex vivo* measures of mitochondrial energetics have detected diminished capacity for basal (Petersen et al. 2003) and maximal (Conley et al. 2000, Kent-Braun and Ng 2000, Short et al. 2005a) mitochondrial ATP synthesis in older adults. When expressing the rate of mitochondrial ATP synthesis relative to mitochondrial content there remains a deficit in older adults suggesting that there is not only a reduction in mitochondrial protein content but also mitochondrial protein quality. These findings appear to be related to physical activity, as sedentary individuals had lower *in vivo* mitochondrial function compared to active individuals (Kent-Braun and Ng 2000, Larsen et al. 2012). It is important to acknowledge that in aging human skeletal muscle, findings of mitochondrial dysfunction are highly equivocal and the disparity between studies is not well discussed. In Table 1 we provide potential confounding variables related to the characteristics of research participants (column A) as well as the use of various measurements of mitochondrial abundance or function (column B). Key differences exist when interpreting data since each measurement in Table 1 assesses different constituents of mitochondrial abundance or function and each method presents key strengths and weaknesses as has been reviewed in detail previously (Lanza and Nair 2010, Perry et al. 2013). One difference could be comparisons between content or maximal activities of enzymes in the mitochondrial matrix (e.g., citrate synthase, HAD) which are completely encoded by nuclear DNA versus proteins involved in oxidative phosphorylation (e.g., cytochrome c oxidase, NADH) that are encoded by both nuclear and mitochondrial genomes. Although analysis of maximal mitochondrial energetics *in vivo* (i.e., ³¹P-MRS) and *ex vivo* (i.e., high-resolution respirometry) are highly correlated (Lanza et al. 2011), subtle discrepancies still exist between different approaches for measuring mitochondrial function *in vivo* (basal vs. maximally stimulated) and *ex vivo* (ATP production vs. oxygen respiration; permeabilized fibers vs. isolated mitochondria). Also, sampling of human muscle tissue from various muscle groups consisting of different recruitment patterns and fiber type composition can create conflicting results between studies. These variables need to be recognized and addressed to properly assess the true age-related phenotype. Globally, when investigations utilize large sample sizes and rigorous control to avoid many of the confounding variables

there appears to be an age-related decline in mitochondrial protein content, quality and function in the quadriceps femoris muscles. These data provide well-founded evidence for perturbations in mitochondrial health and connections to impaired functional capacity during sedentary aging.

Aerobic training is an effective exercise prescription to stimulate markers of oxidative capacity as established in the 1960's (Holloszy 1967), when it was revealed that aerobic exercise of sufficient intensity increased mitochondrial enzyme activity in animal models. Numerous other investigations have confirmed these results, however, few studies in humans have directly investigated if age influences exercise induced mitochondrial adaptations after the same exercise training program. From the few available studies, it appears that mitochondrial molecular regulation and protein content are increased after 12–16 weeks of exercise training, independent of age, suggesting older individuals (<80y) adapt favorably to exercise training (Ghosh et al. 2011, Short et al. 2003). However, the influence of various exercise training programs (i.e., aerobic vs. resistance vs. concurrent training) on mitochondrial and skeletal muscle function (*ex vivo* or *in vivo*) has yet to be determined and warrants investigation. Collectively, these data suggest that exercise can improve or prevent the loss of mitochondrial health during sedentary aging (Figure 2).

3.0 Molecular Regulation of Aging Mitochondria

The mitochondria consist of proteins encoded from both mitochondrial (mtDNA) and nuclear DNA (nDNA). Although mtDNA contains just 27 genes that encode 13 proteins (all within the electron transport chain), 2 ribosomal and 22 translational RNA, proper organelle biogenesis and function require input from both genomes. Several transcription factors and molecular regulators have been highlighted in orchestrating mitochondrial biogenesis and substrate metabolism. The exploration of the molecular regulation of mitochondria has received much attention to gain insight into the etiology of aging mitochondria and associated disease conditions. The family of sirtuins, NAMPT, PGC-1, NRF 1, NRF 2, TFAM as well as metabolite sensors such as AMPK, CAMK and calcium flux play an integral role in maintaining mitochondrial homeostasis as illustrated in Figure 2.

3.1 Sirtuins

The Sirtuin family (SIRT 1–7) is an NAD-dependent histone/protein deacetylase that interacts with transcription factors and cofactors influencing many metabolic pathways (for review see (Guarente 2011, Gurd 2011, Westphal et al. 2007, White and Schenk 2012)). SIRT1 is the most well described Sirtuin due to the favorable impact on targets associated with cellular growth, chromatin remodeling, substrate metabolism and mitochondrial biogenesis. Specifically, the capability of SIRT1 to deacetylate PGC-1, relaying signal transduction for mitochondrial biogenesis, improved substrate utilization and insulin action is particularly relevant in improving the aging phenotype. In addition, SIRT3, which is localized to the mitochondria, is associated with mitochondrial efficiency and ROS production. SIRT3 knockout animals demonstrate hyperacetylation interfering with proper mRNA transcription, elevated levels of ROS and concomitant reduction in ATP synthesis much like many aging models (Ahn et al. 2008, Kim et al. 2010). These data are substantiated from mechanistic studies displaying the ability of SIRT3 to deacetylate and activate MnSOD and glutathione-scavenging pathway enzymes to protect from reactive oxygen species (ROS) during SIRT3 overexpression and caloric restriction (Someya et al. 2010). Elevated ROS emissions from the mitochondria have been implicated in the progression of mitochondrial dysfunction and development of insulin resistance (Fisher-Wellman and Neuffer 2012). Therefore, SIRT 1 and 3 may play a role in mitochondrial health, insulin action and functional capacity with advancing age.

Sedentary older adults contain less SIRT3 content, however, it appears those who perform vigorous endurance exercise are capable of maintaining SIRT3 compared to their younger counterparts (Lanza et al. 2008). Indeed, SIRT3 abundance and activity increase after contractile activity and may be a potential mechanism for improved ATP synthesis, ROS production and insulin action (Gurd et al. 2012). Exploring the upstream regulation of the sirtuin family is essential to fully appreciate how various interventions (i.e., exercise, caloric restriction, medications) mediate the intracellular signaling pathways associated with mitochondrial biogenesis and protein quality.

3.2 Peroxisome proliferator-activated receptor- γ coactivator (PGC)-1 α

In aging human skeletal muscle, there have been observations of either reduced or unaltered levels of PGC-1 (Lanza et al. 2008, Ling et al. 2004). Since PGC-1 is considered the master regulator of mitochondrial biogenesis, lower levels may partially reduce downstream transcription factors as well as mitochondrial content and function. This hypothesis is supported by a diminished capacity to stimulate mitochondrial biogenesis or maintain mitochondrial content during active-aging in animals with genetically altered PGC-1 (Leick et al. 2010). Additionally, animals with overexpressed PGC-1 demonstrate mitochondrial biogenesis and reversal of many age-related diseases including sarcopenia (Wenz 2011, Wenz et al. 2009). Acute and chronic aerobic exercise increase PGC-1 mRNA expression similarly in young and old individuals (Cobley et al. 2012, Short et al. 2003). Older people who have maintained a high level of aerobic exercise for several years had greater protein expression of PGC-1 than sedentary young people yet failed to achieve similar levels as younger people who also maintained high levels of aerobic training (Lanza et al. 2008) suggesting that exercise can mitigate some age-related losses but cannot fully protect the molecular regulation of mitochondrial biogenesis. Skeletal muscle biopsy samples obtained from a unique group of adults over 80y of age revealed that those who engaged in vigorous life-long endurance exercise have greater PGC-1 mRNA expression compared to their healthy counterparts who performed normal activities of daily living (Trappe et al. 2012). Although PGC-1 is not obligatory for exercise induced mitochondrial biogenesis (Leick et al. 2008) it still appears to be a valuable component in the effects of exercise on skeletal muscle and metabolic health with advancing age. Interestingly, an isoform of PGC-1 (PGC-1 β) has been comprehensively demonstrated to be involved in skeletal muscle hypertrophy *in vitro* and *in vivo* (Ruas et al. 2012). PGC-1 β appears to be the primary isoform associated with skeletal muscle growth through the stimulation of IGF-1 and suppression of myostatin, MuRF-1 and Atrogin-1 mRNA expression. Interestingly, previous studies have demonstrated that aerobic (Konopka et al. 2010) and resistance (Kim et al. 2005, Roth et al. 2003, Ryan et al. 2011, Williamson et al. 2010) exercise training reduced catabolic mRNA expression (i.e., FOXO3a, MuRF-1, Atrogin-1 and/or myostatin) with concomitant skeletal muscle hypertrophy. These adaptations could be associated with elevated levels of PGC-1 β as recently revealed but further examination is needed. Elevated PGC-1 isoforms with exercise training in sedentary humans occurs concomitantly with increased mitochondrial protein content, mixed muscle protein synthesis, skeletal muscle hypertrophy and aerobic capacity (Harber et al. 2009b, Harber et al. 2012, Ruas et al. 2012, Short et al. 2004, Short et al. 2003). Collectively, these investigations highlight the advantages of PGC-1 in promoting a healthy aging, skeletal muscle phenotype.

3.3 Mitochondrial transcription factor A (TFAM) and Nuclear respiratory factor (NRF)-1 & -2

Recent research has revealed that PGC-1 relocates to both the mitochondrial and nuclear compartments after a physiological stimulus, such as aerobic exercise, to coordinate mitochondrial biogenesis from both nuclear and mitochondrial genomes (Safdar et al. 2011). Most reports describe PGC-1 by directly acting on NRF-1 & -2 within the nucleus to

stimulate increased levels of mitochondrial transcription factor A (TFAM) followed by import into the mitochondria. However, it appears that PGC-1 can also act independently on NRF-1 and TFAM by binding to the NRF-1 promoter region within the nucleus as well as being complexed with TFAM and the mtDNA D-loop region within the mitochondrial matrix to transcriptionally coordinate nuclear- and mitochondrial-encoded proteins. These studies established an updated paradigm into the mechanisms of how PGC-1 orchestrates mitochondrial biogenesis through key transcriptional regulators NRF-1 and TFAM.

In addition to binding to mtDNA for transcriptional induction of mitochondrial biogenesis, TFAM also has a strong affinity for mtDNA to stabilize and package the genome into a nucleoid structure (for detailed review see (Campbell et al. 2012)). Stabilizing mtDNA appears to be a protective mechanism to prevent damage and/or loss of mtDNA copy number as observed in aging skeletal muscle (Short et al. 2005a). However, in aging brain tissue, TFAM was elevated with a concomitant reduction in mtDNA most likely due to impaired binding of TFAM to mtDNA regions (Picca et al. 2012). Mitochondrial protein turnover via Lon protease, discussed in the next section, is thought to regulate the TFAM:mtDNA ratio to enhance stability and transcription (Matsushima et al. 2010). Exercise is known to increase TFAM and mtDNA number highlighting the potential for improved TFAM-mtDNA binding with chronic exercise. The regulation of transcription, translation and mitochondrial biogenesis is still not completely understood and further research is warranted.

4.0 Skeletal Muscle and Mitochondrial Protein Turnover

In addition to transcriptional regulation, the accretion of new proteins and degradation of older proteins may have a large impact on mitochondrial morphology and function. It is important to note that changes in mitochondrial protein turnover may not always be reflected by expression of mitochondrial proteins. For example, when the rates of *de novo* synthesized proteins are elevated while being matched by the breakdown of older irreversibly modified proteins, no apparent changes in protein abundance can be detected. More importantly, elevated protein turnover (i.e., the replacement of modified and presumably dysfunctional proteins by *de novo* synthesized proteins) may be a strategic mechanism to maintain mitochondrial protein quality and function (Figure 3). This notion is supported by a study demonstrating that life long caloric restricted mice maintained mitochondrial function with age but did not increase mitochondrial abundance (Lanza et al. 2012). Instead of mitochondrial proliferation, caloric restricted mice improved mitochondrial protein quality compared to their ad libitum fed counterparts. These concepts strongly emphasize the need to measure mitochondrial protein synthesis and breakdown as a process to increase or maintain mitochondrial function with age.

4.1 Mitochondrial Protein Synthesis

Infusion of stable isotope tracer into young and older adults demonstrated decreased rates of *in vivo* mitochondrial protein synthesis rate in skeletal muscle of older adults and is accompanied with diminished mitochondrial enzyme activity (Rooyackers et al. 1996). These data provide a feasible connection between the decreased ability to replace mitochondrial proteins in aging skeletal muscle leading to mitochondrial enzymatic dysfunction. Furthermore, mitochondrial protein synthesis rates are initially reduced during middle age, which may be a precursor to the progressive loss of mitochondrial protein abundance and function with older age. Other investigations have confirmed the deficit in mitochondrial protein synthesis rate in older adults (Guillet et al. 2004) while advancements of innovative methodology has allowed for the analysis of individual mitochondrial protein synthesis rates to elucidate specific proteins that may participate in the etiology of aging mitochondrial dysfunction (Jaleel et al. 2008, Lanza et al. 2012).

While increased mitochondrial content is an established adaptation of aerobic exercise, the impact of exercise on mitochondrial protein turnover is not well characterized. One group has demonstrated that acute and chronic aerobic exercise increases mitochondrial protein synthesis rates in younger individuals (Wilkinson et al. 2008). However, the effects of exercise on aging mitochondrial protein turnover have not yet been examined. Due to the clear associations with mitochondrial biogenesis and function, studies are needed to comprehensively elucidate the impact of exercise training on overcoming diminished mitochondrial protein synthesis in aging humans.

4.2 Mitochondrial Protein Degradation

Due to difficulties of properly assessing protein degradation in human skeletal muscle, the literature is equivocal and largely unknown. However, global assessments indicate whole-body protein degradation is reduced in older adults (Balagopal et al. 1997, Henderson et al. 2009). These data, in conjunction with lower rates of mixed muscle (Short et al. 2004), myosin heavy chain (Balagopal et al. 1997) and mitochondrial protein synthesis (Rooyackers et al. 1996), suggest that a low protein turnover in older adults may allow for a reduction in protein quality by accumulation of modified proteins in organelles (i.e., mitochondria) and tissues (i.e., skeletal muscle) creating further dysfunction with age (Figure 3). It is important to note that different skeletal muscle subfractions (Balagopal et al. 1997, Rooyackers et al. 1996, Short et al. 2004, Trappe et al. 2004), fiber types (Dickinson et al. 2010), and individual proteins (Jaleel et al. 2008) have diverse rates of protein turnover and warrant further examination as a component of the age-related loss of mitochondrial and skeletal muscle health. Currently, methods to determine skeletal muscle and mitochondrial protein degradation are not well developed. These key limitations highlight the need for advancement of novel techniques to properly assess protein degradation and propel our understanding of human skeletal muscle biology.

4.2.1 Mitochondrial Dynamics—One particular area of interest regarding mitochondrial turnover is the collaboration of mitochondrial fusion, fission and autophagy (i.e. mitochondrial dynamics) to regulate organelle morphology. Mitochondrial fusion is the combination of outer mitochondrial membrane and subsequent mixing of intramitochondrial components to dilute any damaged mitochondrial DNA or proteins. Additionally, mitofusion proteins also assist in molding the inner mitochondrial membrane cristae, making the collective purpose of mitofusion to prevent the dissipation of mitochondrial membrane potential and thus ATP synthesis. Investigations utilizing animal knockout models of mitofusion proteins have demonstrated diminished mitochondrial function and biogenesis as well as muscle atrophy (Chen et al. 2010).

Conversely, when fusion is no longer possible due to the loss of mitochondrial membrane integrity, fission is responsible for the fragmentation and excision of any altered or damaged mitochondrial components that are subsequently degraded by mitochondrial specific autophagy (i.e. mitophagy) (Seo et al. 2010). Mitochondrial dynamics are essential to maintain normal mitochondrial metabolism, morphology and homeostasis in highly oxidative tissues such as skeletal muscle (Masiero et al. 2009). Therefore, from recent research revealing that select mitofusion and mitofission markers (i.e., mRNA) are reduced in aging human skeletal muscle (Crane et al. 2010), we can infer that mitochondrial turnover is compromised which could partially mediate age-related mitochondrial dysfunction and impaired skeletal muscle health. Interestingly, markers of mitochondrial dynamics are elevated with acute exercise in young individuals (Cartoni et al. 2005, Slivka et al. 2012) suggesting that exercise can increase mitochondrial turnover, which may lead to favorable mitochondrial functional improvements.

4.2.2 Proteolytic Pathways—The autophagy-lysosome and ubiquitin-proteasome (UPP) pathways are two major systems that mediate protein degradation and maintain cellular homeostasis. Autophagy appears to be associated with more bulk protein degradation of large areas and/or organelles, such as mitochondria, that are encapsulated by the phagophore, fused with the lysosome and subsequently broken down to amino acids (Figure 2). Conversely, the UPP is responsible for marking select proteins that are damaged or misfolded with an ubiquitin tail for degradation via the proteasome. Recent research suggests that the UPP may interact with autophagy by assisting the regulation of mitochondrial dynamics and disposal of damaged mitochondrial proteins. Additionally, evidence indicates the role of UPP in regulating cellular homeostasis may be distinct in various skeletal muscle organelles or sub-fractions (i.e. sarcoplasmic, myofibrillar, mitochondrial).

One mitochondrial quality control mechanism is the ATP-stimulated Lon protease located in the mitochondrial matrix (Figure 2). Lon protease is believed to be an integral factor in the degradation of oxidatively damaged mitochondrial proteins (Bota and Davies 2002). In aging models, Lon protease is reduced and therefore hypothesized to play a role in the development of mitochondrial dysfunction in older tissues (Bota et al. 2002, Lee et al. 1999). Another mitochondrial quality control pathway is autophagy, as evidenced by the maintenance of mitochondrial function in the liver of older transgenic mice compared to wild type mice (Zhang and Cuervo 2008). Similarly, overexpression of autophagy proteins in human umbilical vein endothelial cells appears to remove damaged mitochondrial proteins when challenged with reactive oxygen species in vitro (Mai et al. 2012). Data in human skeletal muscle are limited but recent studies have observed no measurable differences between young and older individuals at the mRNA level for markers of UPP or autophagy (Fry et al. 2012a). It is interesting to note that mRNA of UPP was elevated and/or autophagy reduced in humans undergoing accelerated atrophy (i.e. >80y old (Raue et al. 2007, Williamson et al. 2010), para- (Fry et al. 2012b) and hemiplegia (von Walden et al. 2012)). Development of dynamic assays to measure protein degradation specific to the mitochondrial and myofibrillar proteins are needed to provide a direct functional connection to protein metabolism and age.

Acute aerobic exercise appears to increase mRNA expression of proteolytic pathways within mixed muscle homogenates (Harber et al. 2009a, Harber et al. 2010, Louis et al. 2007, Pasiakos et al. 2010). These data suggest that the molecular induction of protein degradation is elevated by acute exercise, most likely providing amino acids for *de novo* synthesis (Balagopal et al. 2001) and myocellular remodeling that leads to improved contractile function after chronic exercise (Harber et al. 2004, Harber et al. 2009b, Harber et al. 2012, Trappe et al. 2001). Interestingly, exercise training programs that improve skeletal muscle size and function in adults (<80 y) observed reductions in proteolytic markers (Konopka et al. 2010, Williamson et al. 2010), most likely shifting protein balance in favor of skeletal muscle protein accretion. Collectively, these data reveal the differences between transient alterations and chronic adaptations in proteolytic machinery highlighting the need for additional investigations examining protein turnover to substantiate the link to myocellular and mitochondrial function after acute and chronic exercise.

5.0 Insufficient Antioxidant Capacity and Oxidative Damage

Reactive oxygen species (ROS) are molecules containing one or more unpaired electrons mainly produced from complex I and III in the ETC. These byproducts are normally detoxified by antioxidants (e.g. MnSOD, CuZnSOD, catalase) but when they are generated in excess of antioxidant capacity they can irreversibly modify (i.e. damage) lipids, proteins, and DNA. Due to inconsistent findings on the antioxidant capacity in older adults (Ghosh et

al. 2011, Gianni et al. 2004, Leeuwenburgh et al. 1994, Pansarasa et al. 2000) there is a strong need to determine antioxidant capacity at the systemic, skeletal muscle and mitochondrial compartments to fully identify where these defense mechanisms fail and where development of therapies should be focused to ameliorate oxidant damage and cellular dysfunction with age.

Damage to mtDNA is suspected to occur easily due to the proximity near the ETC and lack of protection by histones. The combined effects of reduced protein turnover and excess ROS emission create an environment conducive in aging skeletal muscle for the accumulation of oxidatively damaged mtDNA and contractile proteins (Figure 3). Accrual of mtDNA damage may then allow production of dysfunctional proteins within the ETC, further exaggerating ROS emission and oxidative damage. This scenario is considered the mitochondrial theory of aging first hypothesized by Harman in 1972 (Harman 1972). However, it is important to note that the appearance of mtDNA deletion/mutations in human skeletal muscle is relatively small, with data indicating that mtDNA deletions may reach levels of physiological significance only in adults greater than 80y old (Bua et al. 2006, Chabi et al. 2005, Kopsidas et al. 1998, Kovalenko et al. 1997) although many age-related changes including sarcopenia begin much earlier. When mtDNA deletions are present in single muscle fibers they appear red and ragged, leading to fiber atrophy and eventually fiber loss (Bua et al. 2006). Moreover, mtDNA deletions and fiber atrophy are more prevalent in fast MHC II fibers, which is consistent with age-related loss of fast fiber composition and contractile properties. The pattern of mtDNA deletion mutations, fiber atrophy and fiber loss provides a clear relationship between mitochondria and skeletal muscle atrophy.

Data from mitochondrial bioenergetics supports the role of altered mitochondrial membrane potential in mediating excess levels of oxidant production (Fisher-Wellman and Neuffer 2012). The concept is based on redox biology of mitochondria where over nutrition (i.e. increased supply) and/or reduced physical activity (i.e. reduced demand) appears to create a buildup of protons thus creating a high membrane potential that could cause a cessation of electron flow through the ETC. The disruption in electron flow is thought to increase oxidant production by acting as a release valve to dissipate the elevated membrane potential. Any mechanism to allow protons to flow back to the mitochondrial matrix (ATP synthesis or uncoupling) will also help relieve membrane potential and therefore oxidant production. This notion is supported by findings that life long caloric restriction attenuates H₂O₂ emission by eliminating excess energy intake (i.e. supply) and mitigates the accumulation of post-translational modifications, especially oxidation and deamidation, while concomitantly maintaining mitochondrial energetics with age (Lanza et al. 2012). These novel data are available due to recent advancements where proteome quality can be assessed by the evaluation of post-translational modifications using tandem mass spectrometry. Not only can this innovative approach comprehensively detect global protein modifications, it can also determine which amino acid residues are most commonly modified. Implementing proteome analysis of post-translational modifications may provide a platform to develop and test innovative strategies to reduce the accumulation of modified and functionally impaired proteins.

In addition to caloric restriction, physical activity levels play a strong role in modulating skeletal muscle quality as sedentary older individuals have a noticeable decline in antioxidant capacity with concomitant oxidative damage compared to age-matched active individuals (Safdar et al. 2010). Oxidative modifications can affect action potential propagation, ETC complexes, calcium transport/regulation, and myosin and actin interaction; collectively reducing skeletal muscle function in rodent models (Fulle et al. 2004, Rossi et al. 2008). In a unique model of muscle dysfunction, individuals with chronic

obstructive pulmonary disease were characterized with increased oxidative damage that was negatively correlated with aerobic capacity and isometric skeletal muscle force production (Barreiro et al. 2009). These data are supported in older adults as elevated oxidative damage has been correlated with diminished functional capacity (Howard et al. 2007, Semba et al. 2007a) and increased risk of mortality (Semba et al. 2007b). Therefore, there are implications that excess ROS may be an underlying characteristic in the progression of muscle atrophy by introducing oxidative damage that can negatively affect the functional capabilities of older adults.

6.0 A role for epigenetic regulation of mitochondrial and contractile proteins

The age-related decline in transcription (i.e., mRNA expression) and translation (i.e., protein synthesis) are apparent in the loss of mitochondrial and contractile protein quality. The underlying mechanisms for reduced transcription include epigenetics, which also has a responsibility in shaping the aging phenotype in response to environmental influences like physical activity and diet. Common epigenetic mechanisms are modifications to DNA and/or histones (i.e., acetylation, methylation). Changes in the methylation and acetylation status can modify chromatin structure, which in turn alters the binding capability of transcription factors to create mRNA (Figure 4). Epigenomic research has large implications in improving our understanding of the aging mitochondrial and contractile dysfunction but is currently at its nascent stages.

It appears with older age there is an increase in DNA methylation of promoter regions for genes involved in oxidative phosphorylation and the level of DNA methylation is inversely correlated with gene expression (Ling et al. 2007, Ronn et al. 2008). These data suggest that altered methylation could be involved in reduced mitochondrial mRNA expression and protein synthesis observed in older adults. More expansive investigation is necessary to determine key promoter regions that may be differentially methylated. Furthermore, alterations to mitochondrial function may provide feedback to influence epigenetic regulation of mitochondrial and nuclear genome.

In addition to mitochondrial regulation, epigenetic alterations also appear to influence key skeletal muscle contractile proteins. The balance between histone acetylation and deacetylation changes in concordance with shifts in myosin heavy chain composition during unloading (Pandorf et al. 2009). This is of particular interest when exploring the mechanisms mediating changes in MHC composition of aging skeletal muscle that occurs with concomitant reductions in size and contractile performance of fast, MHC IIa fibers (Lexell et al. 1983, Trappe et al. 2003). Fast myosin light chain isoforms, thought to influence contractile function, were found to be negatively correlated with promoter methylation (Donoghue et al. 1991) suggesting that methylation may inhibit fast fiber performance as observed in older adults.

Currently, exercise seems to be one of the most impactful stimuli that alters skeletal muscle physiology (i.e., MHC and mitochondrial protein abundance and function (Balagopal et al. 1997, Coggan et al. 1992, Ghosh et al. 2011, Harber et al. 2009b, Harber et al. 2012, Konopka et al. 2011, Short et al. 2005b, Short et al. 2003)) and serves as a countermeasure to many negative consequences of sedentary aging. However, the effects of exercise and age on epigenetic regulation of mRNA expression and skeletal muscle adaptations are relatively unknown. Acute aerobic exercise can induce histone modifications that may mediate chromatin remodeling and disassociations with transcription factors implicated in substrate metabolism (McGee et al. 2009, McGee and Hargreaves 2004). Moreover, acute and chronic contractile activity increased SIRT1 deacetylase activity within the nucleus which is most

likely related to increased PGC-1 mRNA (Gurd 2011, Gurd et al. 2011). An eloquent study (Barres et al. 2012) recently revealed that acute aerobic exercise of sufficient intensity can alter global and promoter specific methylation leading to changes in mRNA expression related to mitochondrial and substrate regulation in young adults. More investigations like Barres et al. are needed to clearly determine the influence of age and chronic exercise on the epigenetic regulation of global and specific promoter regions related to mitochondrial (e.g, PGC-1, SIRT1, TFAM) and contractile proteins (e.g. MHC, MLC, actin, troponin) (Figure 4). Discovering the epigenetic modulation of skeletal muscle has vast potential to facilitate the development of novel therapies to improve protein quality with age.

Perspectives on Aging Skeletal Muscle and Mitochondrial Health

The common loss of mitochondrial and skeletal muscle volume, function and quality is an attractive relationship to help explain the comorbidities associated with aging. More research is needed to confirm that the loss of mitochondria health undermines sarcopenia, especially due to the variability within human studies. Some inconsistency seems to be related to different physical activity and lifestyle choices between subjects. This variability may partially be explained by the implementation of epigenetic research to gain comprehensive insight into the molecular regulation of aging in sedentary and physically active individuals. Exercise training and physical activity remain effective strategies to attenuate the development of mitochondrial and skeletal muscle dysfunction but many questions remain unanswered. Moreover, the role of chronic caloric restriction requires controlled studies that balance the potential adverse effects due to loss of lean mass and energy balance versus beneficial effects associated with reduced oxidative damage to proteins and DNA. In many aging populations, adherence or ability to participate in exercise is limited due to orthopedic or disease limitations (i.e. heart failure, chronic obstructive pulmonary disease) which warrants exploration of other therapies (i.e., caloric restriction, diet composition, non-traditional exercise, etc.) to help prevent the negative health consequences and elevated healthcare costs related to sedentary aging.

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Highlights

1. A temporal and functional connection exists between the decline of mitochondrial and skeletal muscle health with age.
2. Lower mitochondrial mRNA expression, protein abundance and synthesis rates promote the decline of mitochondrial function.
3. Reduced mitochondrial function may impede energy demanding processes such as protein turnover.
4. Decreased protein turnover results in lower protein quality and function in aging skeletal muscle.

Figure 1. Reduced Mitochondrial and Skeletal Volume, Quality and Function with Sedentary Aging

Sedentary aging is associated with the decline of mitochondrial and skeletal muscle volume, quality and function. The casual link between the loss of mitochondrial homeostasis and sarcopenia is unknown, however, both appear with advancing age and are associated with the loss of functional capacity and corresponding increases in comorbidities and annual healthcare costs. Exercise and physical activity are effective prescriptions to attenuate the negative consequences of sedentary aging illustrated in Figure 1.

Figure 2. Mitochondrial Adaptations to Aerobic Exercise Training

With contractile activity, elevated levels of metabolic byproducts (i.e., NAD, AMP, Ca^{++} , ROS, etc.) provide a stimulus for increased molecular regulators of mitochondrial transcription, replication and dynamics (i.e., NAMPT, SIRT-1, PGC-1, NRF-1, -2, TFAM, MFN-1, -2, FIS1, DRP1). Collectively, these alterations promote the increase in mitochondrial protein turnover allowing for the degradation of damaged proteins and *de novo* synthesis of new functional proteins. Overall, the elevated rate of mitochondrial protein turnover suggests an improvement in the quality of mitochondrial proteins for enhanced ATP production and lower reactive oxygen species (ROS) emission. Enhanced mitochondrial function may augment myocellular remodeling, skeletal muscle anabolism and functional capacity in older adults.

Figure 3. Effect of Aging and Exercise on Protein Damage and Quality

With aging, excess reactive oxygen species (ROS) are produced from mitochondria with a concomitant reduction of protein degradation and synthesis (i.e., protein turnover). The combination of elevated ROS and reduced protein turnover can lead to the accumulation of damage to proteins resulting in reduced protein quality and function. We hypothesize that exercise training can attenuate age-related production of ROS and stimulate protein turnover, in turn, degrading oxidatively damaged proteins and replacing with newly synthesized, functional proteins.

Figure 4. Epigenetic Regulation of Mitochondrial and Contractile Proteins

Epigenetic control of mitochondrial and contractile proteins may improve our knowledge on the development of the aging phenotype. During sedentary aging, chromatin is condensed by altered levels of acetylation and/or methylation that prevent binding of transcription factors. With exercise, modifications of histones (indirect modification) and/or DNA (direct modification) may allow an open chromatin structure where transcription factors can bind to DNA promoter regions for mRNA transcription to occur. The shifts between condensed chromatin during sedentary aging and open chromatin with exercise are partially mediated by DNA and histone modifying enzymes: DNA methyl transferases (DNMT), histone acetyl transferases (HATs), histone methyl transferases (HMTs), histone deacetylases (HDACs) and histone demethylases (HDMs). The schematic was adopted from Zwetsloot et al. (Zwetsloot et al. 2009) and altered to reflect our perspective on epigenetic control of mitochondrial and contractile protein quality.

Table 1
Divergent subject characteristics and analytical techniques may contribute to discrepancies regarding aging mitochondrial health

A. Subject Characteristics	References	B. Analytical Techniques	References
Age	(Bua et al. 2006, Chabi et al. 2005)	Maximal enzyme activity	(Barrientos et al. 1996, Coggan et al. 1992, Holloszy 1967, Holloszy and Coyle 1984, Zucchini et al. 1995)
Sample Size	(Chretien et al. 1998, Short et al. 2005a)	Protein abundance	(Lanza et al. 2008)
Mobility or orthopedic limitations	(Boffoli et al. 1994, Joseph et al. 2012, Lezza et al. 1994, Safdar et al. 2010)	mtDNA copy number and mutations	(Aiken et al. 2002, Short et al. 2005a)
Exercise/Physical activity history	(Barrientos et al. 1996, Lanza et al. 2008, Proctor et al. 1995)	mRNA expression	(Short et al. 2003, Wright et al. 2007)
Sarcopenia/Frailty	(Moore et al. 2010, Waters et al. 2009)	Electron microscopy	(Conley et al. 2000, Hoppeler et al. 1985, Howald et al. 1985)
Adiposity	(Karakelides et al. 2010)	<i>In vivo</i> function (³¹ P-MRS)	(Amara et al. 2007, Conley et al. 2007, Kent-Braun and Ng 2000, Lanza et al. 2011, Lanza and Nair 2010, Petersen et al. 2003)
Comorbidities (i.e., insulin resistance, COPD, etc.)	(Barreiro et al. 2009, Ghosh et al. 2011, Petersen et al. 2003)	<i>Ex vivo</i> function	(Lanza et al. 2011, Lanza and Nair 2010, Picard et al. 2010, Picard et al. 2011, Rasmussen et al. 2003a, 2003b)
Diet and medications	(Fisher-Wellman and Neuffer 2012, Lanza et al. 2012, Robinson et al. 2009)	Skeletal muscle investigated	(Amara et al. 2007, Houmard et al. 1998, Larsen et al. 2012)

The study of aging mitochondria presents inconsistent findings potentially associated with (A) variability in subject characteristics and (B) different analytical techniques to assess mitochondrial abundance or function within skeletal muscle. To examine the true age-related decline in mitochondrial health, descriptive characteristics listed in (A) need to be comprehensively discussed as these variables may each independently affect mitochondrial health. Moreover, the different analytical techniques listed in (B) are all highly but each approach studies different components of the mitochondria (i.e., citrate synthase enzyme activity vs. *ex vivo* ATP production in isolated mitochondria) and therefore may be a contributor to the equivocal findings between studies. Collectively, both subject characteristics and analytical techniques need to be considered when interpreting data describing the aging mitochondrial phenotype. References provided utilize or discuss the associated subject characteristic or analytical technique.