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Skeletal muscle aging and the mitochondria

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Abstract

The decline in human muscle mass and strength (sarcopenia) is a hallmark of the aging process. A growing body of research in the areas of bioenergetics and protein turnover has placed the mitochondria at the center of this process. It is now clear that unless an active life style is rigorously followed, skeletal muscle mitochondrial decline occurs as humans' age. Increasing research on mitochondrial biology has elucidated the regulatory pathways involved in mitochondrial biogenesis, many of which are potential therapeutic targets, and highlight the beneficial effects of vigorous physical activity on skeletal muscle health for an aging population.

Keywords

sarcopenia; mitochondria; protein metabolism; protein synthesis; aging

Human aging and sarcopenia

Sarcopenia (see Glossary) occurs across species including *C. elegans*, drosophila, rodents and primates [1–3]. Skeletal muscle comprises roughly 40–50% of total body mass in humans and declines of 3–10% per decade after the age of 25 [4,5] substantially impair mobility, locomotion and quality of life [6]. Increasing evidence places a decline in mitochondrial content and function at the center of this process. It is now well documented that the rates of synthesis of mitochondrial, myosin heavy chain (a key contractile protein) and mixed muscle protein are decreased with age, along with skeletal muscle oxidative capacity [7,8]. The involvement of declining mitochondrial function on age-related changes in skeletal muscle has been extensively investigated as a means to understand the cause of sarcopenia. However, there is vigorous research and debate about the exact mechanisms by which skeletal muscle aging affects mitochondria, and conversely, aging mitochondria affect skeletal muscle function. This review discusses current findings in the area of muscle mitochondria and aging and how age-related changes in oxidative capacity, protein turnover and mitochondrial dysfunction contribute to aging and sarcopenia.

Does mitochondrial function decline with age?

The maximal rate at which an individual consumes oxygen ($\text{VO}_{2\text{max}}$) declines with age, even after correcting for losses in lean mass [4,5], and resting oxygen (O_2) consumption also shows an age-related decline when corrected for lean mass [9]. Those data along with the knowledge that sedentary older people have a lower arterio-venous difference in the

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extraction of oxygen, compared to younger individuals at $\text{VO}_{2\text{max}}$, suggest that either muscle mitochondrial function or content is reduced as people age [10]. However, indirect calorimetry is a whole body measurement that is affected by changes other than muscle mitochondrial function or content, such as declines in stroke volume [1–3,11]. Muscle specific measurements such as magnetic resonance spectroscopy (MRS) and biopsy are therefore necessary to implicate a decline in mitochondrial content or function as a factor contributing to the age-related decline in the ability to consume oxygen.

Muscle biopsy samples have indicated that certain measures of mitochondrial content decline with age as assessed by electron microscopy, mitochondrial DNA (mtDNA) copy number, proteomics or the activities of key TCA cycle enzymes such as citrate synthase [4,5,12–17]. Those data are strengthened by a declining maximal ATP production rate (MAPR) in older individuals whether measured from biopsy samples by firefly luciferase, or *in vivo* by MRS (Table 1). Moreover, resting state (basal) ATP production rate measured by MRS is also lower in older individuals [19]. However, regarding MAPR there are contradictory results in the literature, which may be related to the skeletal muscle group studied or due to differences in the participant's activity levels (Table 1). Together those measurements, which isolate the muscle from the whole body, along with declining skeletal muscle protein synthesis (an ATP consuming process) place ATP production rate as a possible mechanism for age related sarcopenia. However, in humans the contribution of age directly on mitochondrial content might be secondary to reduced physical activity [7,8,16] and insulin resistance [4,5,18], both of which are increasingly common as individual's age. As aerobic exercise is a powerful stimulus for increasing and maintaining high levels of mtDNA, ATP production rate, and levels of oxidative enzymes [9,16,19,20].

Data from studies on rats show the decline in enzyme activities with age is more pronounced in slow-twitch fibers [10,15,21]. A recent study investigating the mitochondrial electron transport chain (ETC) showed the same reduction in enzyme activity and respiratory capacity in atrophied slow-twitch fibers but greater atrophy was present in fast-twitch fibers without changes in respiratory capacity [6,22]. Those data suggest stark differences in fiber type responses to aging and that any one measurement of mitochondrial function cannot predict muscle atrophy. While data from humans support fiber type differences in enzyme activities [23], muscle recruitment patterns might also be an important factor. Recently Larsen and colleagues [24] used MRS to examine if aging affects muscle MAPR differently in various muscle groups in humans. Based upon the aforementioned studies they hypothesized that a reduction in MAPR would be present in muscles recruited for moderate to high intensity exercise, such as the vastus lateralis (VL), as this type of activity and transcripts of key contractile proteins declines most with age [25,26]; but not in muscles where exercise intensity has minimal recruitment, such as the tibialis anterior (TA). Compared to younger matched controls, older sedentary adults had lower MAPR in the VL, but not the TA, suggesting that an age effect on skeletal muscle MAPR is impacted by both the frequency of recruitment and intensity of exercise in the elderly [26]. Interestingly, moderate short-term (four-month) [19] aerobic exercise and 3-months of resistance exercise [27] reversed the age-related decline in muscle protein synthesis in the VL, and those elderly individuals who undergo regular vigorous exercise long-term (>4yrs) maintain their mitochondrial content [16]. However, those studies have not distinguished between the effects of aerobic and resistance exercise on the synthesis rate of individual muscle proteins (e.g. mitochondrial vs. myofibrillar). Finally, it is also important to distinguish between older adults (40 to 70 years) and advanced age (>80 years). Much tissue heterogeneity and fiber type grouping occurs with advanced age due to repeated cycles of denervation and the accumulation of fibers that failed to be reinnervated [28,29].

Mechanisms of reduced mitochondrial function and aging

Maintaining mitochondrial protein content

Mitochondrial oxidative capacity is determined by the abundance of mitochondrial proteins and the functional ability of those proteins to produce ATP per unit mass of protein. Mitochondrial content is an important measure of oxidative capacity since the activities of mitochondrial enzymes normally scale proportionally, although a decline in the quality of muscle proteins can affect their function. Protein degradation, by autophagy and the proteasome pathway, together with the synthesis of new proteins may improve the functional quality of skeletal muscle mitochondria [30].

Maintaining the mitochondrial proteome requires the coordinated expression of both nuclear and mitochondrial encoded genes that are translated from amino acyltRNA in the sarcoplasm and mitochondrial reticulum (Figure 1). The mechanisms of protein import and mitochondrial function during aging have previously been reviewed [31], yet it should be noted that protein import machinery increases following 7-days of electrical stimulation, a model of endurance exercise in rat skeletal muscle [32]. Mitochondrial proteins with minor oxidative damage can be degraded by the Lon protease [33], while extensive damage with a loss of membrane potential stimulates fission of the membrane and transport to the lysosome for degradation by mitophagy [34]. A decrease in Lon content of skeletal muscle of older mice has been identified [35], and may contribute to the buildup of oxidative damage of mitochondrial proteins with age.

Mitochondrial biogenesis is under transcriptional regulation by regulatory factors such as peroxisome proliferator-activated receptor- γ coactivator 1 α (PGC-1 α) and downstream transcription factors such as nuclear respiratory factor 1 and 2 (NRF-1 and NRF-2) and mitochondrial transcription factor A (TFAM). However, differences in polyribosome formation or activation of translational machinery in response to exercise and other stimuli can regulate protein synthesis independent of transcription [36]. Transient changes in mRNA content occur following acute stimuli, such as exercise, while changes in mitochondrial protein content occur over longer periods [37]. Additionally, matched changes in protein synthesis and degradation form new mitochondrial proteins (biogenesis) with no change in content, but may maintain the quality and functional integrity of mitochondrial proteins because timely degradation of irreversibly (damaged) modified proteins and their replacement by *de novo* synthesized proteins likely maintains mitochondrial function [30]. Thus, mitochondrial biogenesis can occur independently of changes in protein content and increased protein turnover is beneficial when oxidative damage to mitochondrial proteins is high.

Mitochondrial protein content falls with age

Mitochondrial content declines with age in sedentary individuals when measured as enzyme activities or quantitative proteomics [5,12,16]. While endurance training can correct the age related decline in enzyme activities or protein content in older individuals [38], studies linking those results to clinically relevant outcomes are currently lacking. Further studies on advanced age (>80 years) while few in number, suggest that training might not have the same effect [39–41].

The rate of synthesis of mitochondrial proteins is measured by the incorporation of amino acid tracers into mitochondrial protein, over a given time period. The first study to measure mitochondrial protein synthesis rates in young, middle aged, and older individuals (average ages 24 ± 1 , 53.5 ± 2 and 72 ± 3 years respectively) *in vivo* found a 40% decline with age, but all the changes occurred by middle age with no further decline with old age [12]. It remains to be determined whether those with higher mitochondrial protein synthesis

maintain mitochondrial function with age. Subsequent measurements in humans of mitochondrial protein synthesis in response to amino acids and insulin found a reduced stimulatory response in the elderly, possibly due to a defect in phosphorylation of S6K1 [42]. A lower synthesis rate and blunted response to stimulus extrapolated over longer periods of time may explain the decrease in mitochondrial content. Interestingly, the only cumulative mitochondrial synthesis rate measurement published to date is a six-week study on mice that showed an increase in mitochondrial protein synthesis with age, irrespective of whether the mice are fed ad-libitum or 40% caloric restriction [43]. At this time the reason for such differences in results between studies are unknown. Methodological differences may account for the discordant results, because all human studies to date have used a primed continuous infusion to measure protein synthesis, while the approach used by Miller et al. in mice utilized a cumulative six-week measurement. It remains to be determined what contribution degradation of proteins with isotope makes to a long-term synthesis measurement. Further, species-specific differences might also contribute. For example humans reach adulthood followed by a long period of aging compared to rodents, which have a relatively shorter aging period after adulthood is reached. Taken together, the results suggest that aging leads to a decrease in mitochondrial content that can be reversed in older adults by exercise training. However, there is likely an age at which exercise may no longer be beneficial, but research in this area is currently lacking.

Mitochondrial protein degradation

The degradation rate of mitochondrial proteins is much more difficult to measure than synthesis rates. Stable isotopes can be used to determine protein degradation for the whole body [44], across tissue beds [45] and mixed skeletal muscle proteins [46], although none of these methods have addressed the recycling of stable isotopes that is known to occur *in vivo*. Moreover there are no methodologies currently available to measure the degradation rates of protein sub fractions or individual proteins.

Protein degradation by the autophagy-lysosome and ubiquitin-proteasome pathways is important to maintain mitochondrial function and fits a model that decreased protein turnover with age leads to the accumulation of damaged proteins with decreased function (Figure 2). Mitochondrial proteins are particularly susceptible to damage due to their proximity to reactive oxygen species (ROS) formation. The ubiquitin-proteasome and Lon protease pathways catalyze the selective removal of damaged proteins within the mitochondria whereas the autophagy-lysosome pathway degrades larger volumes of mitochondrial proteins or membrane (termed mitophagy). A decrease in proteolysis pathways with age has been detected in various tissues including drosophila flight muscle [47], mouse liver [48], mouse skeletal muscle [49], rat skeletal muscle [50] and human fibroblasts [51]. Transgenic model systems revealed that increasing autophagy related proteins can improve mitochondrial function during mitochondrial damage or aging. Damaged mitochondrial proteins are removed by mitophagy in human umbilical vein endothelial cell cultures and overexpression of ATG5, ATG7 and LC3B protected mitochondrial function following ROS induced damage [30]. Further, transgenic mice that maintain the autophagy protein lysosome-associated membrane protein 2A (LAMP2) in liver have restored autophagy and mitochondrial function with age, compared to wild type [48]. Similar data from human skeletal muscle are lacking and often limited to static markers of autophagy [45,52] which cannot determine flux or specificity to mitochondria. Future studies are needed to investigate changes to mitochondrial protein degradation rates with aging and exercise.

Key mitochondrial signaling proteins and aging

The close relationship between aging, mitochondrial function and metabolic disorders has received much attention and three proteins have emerged as key metabolic signals: PGC-1 α , silent mating type information regulation 2 homolog sirtuin 1 (SIRT1) and AMP activated protein kinase (AMPK).

The role of PGC-1 α —PGC-1 α can stimulate the formation of new mitochondria in cell culture and is an important regulator of exercise tolerance and mitochondrial adaptations to aerobic exercise in rodents [4,53] and humans [16]. Yet, PGC-1 α is not mandatory for exercise adaptations as shown by muscle specific knockout animals showing increased mitochondrial protein content following aerobic training [54]. Overexpression of PGC-1 α in the skeletal muscle of mice attenuates mitochondrial dysfunction at 22 months of age [55]. Similarly, overexpression of PGC-1 α decreases proteolysis in myotube cultures and attenuates atrophy in denervated mouse skeletal muscle [56]. Additionally, PGC-1 α knockout mice have impaired activation of the unfolded protein response [57], which is an important pathway to repair or remove damaged mitochondrial proteins [31]. Thus, PGC-1 α has important roles in regulating mitochondrial proteostasis through promoting the expression of new mitochondrial proteins and maintaining protein health in response to stress such as aging or exercise.

The effects of age on PGC-1 α expression are not clear and are likely due to differences in sarcopenia that are possible in rodent models compared to humans. Some groups have reported lower skeletal muscle PGC-1 α content between age groups in ~70 year old humans (mRNA [58]) and 36 month rats (protein [59]), but not in 24 month mice (mRNA and protein [43]). Interestingly, endurance exercise training promotes PGC-1 α protein content in skeletal muscle of 34 month rats [60] and 59–76 year old humans [16]. It will be important to understand how aging alters the ability for PGC-1 α to regulate the health of mitochondrial proteins.

The role of SIRTUINS—Sirtuins are NAD⁺-dependent protein deacetylase enzymes whose activation can attenuate age related metabolic disorders [61] without changes in longevity [62], in mice. SIRT1 has received much attention due to its potential role in mediating the health benefits of resveratrol supplementation [63] and as a potential pharmaceutical target for treatment of metabolic disorders. SIRT1 colocalizes with PGC-1 α within mitochondria [64] and their coordinated activity appears to be downstream of AMPK in response to exercise or nutrition stress [65]. Much research has focused on SIRT1 being able to promote gene expression via its histone deacetylase activity, however other sirtuins are located throughout cells and have various targets and metabolic regulation. Indeed, mice with depleted hepatic mitochondrial SIRT3 have hyperacetylation of mitochondrial proteins and increased susceptibility to metabolic disorders, upon high fat feeding [66]. Further, skeletal muscle SIRT3 knockout mice have decreased whole body oxygen consumption and increased ROS damage, and C2C12 myotubes lacking SIRT3 have decreased mitochondrial oxygen consumption compared to wild-type mice [67]. In human aging, sedentariness is associated with reduced expression of SIRT3 in skeletal muscle, but vigorous long-term exercise enhances SIRT3 expression and eliminates differences between young and older individuals [16]. Further research is required to elucidate the role of sirtuins in regulating mitochondrial function in humans with aging and chronic disease.

The role of AMPK—One mechanism by which AMPK increases energy production is through increased mitochondrial biogenesis, and decreased basal activation of AMPK may contribute to the loss of mitochondrial content with age [68]. The response of AMPK to nutrient or exercise stimuli may also be blunted with aging. In support, older rats showed

lower AMPK activity following acute or chronic nutrient stimuli or 5-days of aerobic exercise [69]. Such blunted signaling may lead to attenuated mitochondrial protein synthesis and contribute to accumulation of damaged mitochondrial proteins with age.

There is an apparent paradox for AMPK to promote mitochondrial biogenesis during energy stress. AMPK activation restricts global protein synthesis and skeletal muscle cell growth through inhibition of mammalian target of rapamycin (mTOR) Complex 1 (mTORC-1) [70], yet AMPK activation also increases mitochondrial enzyme content of skeletal muscle [71]. Protein synthesis requires ATP and is decreased with AMPK activation, thus AMPK must inhibit global protein synthesis while simultaneously increasing mitochondrial protein synthesis. It is likely that AMPK coordinates with PGC-1 α and sirtuin activity for selectively increasing mitochondrial protein synthesis, while decreasing overall protein synthesis during energy stress [65].

Mitochondrial dysfunction

The concept of mitochondrial dysfunction and our understanding of mitochondrial biology are increasingly becoming more defined as researchers improve isolation and measurement methodologies. By mitochondrial dysfunction most investigators refer to either reduced maximal ATP production from *in vitro* methods such as the bioluminescence approach [5], high-resolution respirometry [72], or *in vivo* methods such as MRS [24] or reduced resting ATP production rate by MRS [18].

Early reports of mitochondrial decline in aging found a progressive 5% reduction per decade in mtDNA abundance and ATP production rate leading us to hypothesize that mtDNA abundance may drive the aging process in skeletal muscle (Fig 3.) [5,16]. Reduced MAPR with age was also observed using both *ex vivo* measurements [5] and *in vivo* MRS on sedentary individuals [17,18]. Importantly however, older individuals have a similar magnitude increase in aerobic capacity following endurance training as younger individuals [20].

Recently, more attention has turned to the population of mitochondria that is isolated, during these measurements. First, micrographs of aged skeletal muscle often show enlarged and disrupted mitochondria [13], suggesting that mitochondrial isolation procedures of biopsy samples might bias towards only the healthy mitochondria, potentially masking an age effect. Second, there are two distinct populations of skeletal mitochondria located in the subsarcolemmal (~20% of total mitochondrial content) and intermyofibrillar regions (~80% of total mitochondrial content) that are not uniformly released, due to differences in isolation technique and detergents [73,74]. The population is critical because subsarcolemmal mitochondria are thought to play a more important role in maintaining the cellular processes of skeletal muscle and are possibly disproportionately affected by sedentary behavior [38].

A recent study of Picard et al [72] examined the differences in mitochondrial function on isolated mitochondria, where both the subsarcolemmal and intermyofibrillar mitochondria were isolated and then compared to permeabilized muscle fibers from the same sample. The permeabilized muscle fiber preparation preserves the mitochondrial reticulum along with any structural interactions and morphology that might be important. Picard et al found that mitochondrial function was decreased in both isolated mitochondria and permeabilized fibers with an exaggerated aging effect in isolated mitochondria. Those data confirm that an age effect on mitochondrial function is occurring, however, the *in vivo* functional significance might be less than previously thought. Importantly, the results suggest that sarcopenic muscle demonstrates an impaired ability to tolerate the stresses of isolation procedures and perhaps might be more susceptible to other stress such as ROS.

Oxidative damage as a cause of mitochondrial dysfunction in aging muscle

An age related increase in oxidative stress mediated by increases in ROS is one process that, if not matched by the activity of endogenous antioxidants, could cause mitochondrial dysfunction by damaging cellular lipids, proteins and nucleic acids. The literature on human skeletal muscle strongly supports a positive correlation between age and oxidative damage to levels of lipid peroxidation, protein carbonyl content and 8-oxo-deoxyguanosine (8-oxo-dG), a measure of DNA oxidation [75,76]. mtDNA is thought to be sensitive to oxidative damage due to its lack of histones and proximity to ROS produced by the electron transport chain. Indeed mtDNA abnormalities increase with age and are associated with sarcopenia [77–80]. But because of the heterogeneity of mtDNA sequence, cross sectional studies are not conclusive on mtDNA mutation and aging. Whole body overexpression of catalase in the mitochondria of mice prevents the deleterious metabolic impairments associated with aging [68]. Interestingly, older humans with sarcopenia have similar endogenous antioxidants content (superoxide dismutase, catalase, and glutathione peroxidase) compared to younger individuals [81] suggesting that more endogenous antioxidant activity is necessary to combat the age associated increase in ROS production.

Establishing causation of ROS induced damage to mitochondria that in turn affects skeletal muscle aging is a more difficult question to answer. The leading hypothesis purports that ROS induced oxidative damage to proteins in the electron transport chain will cause increased leakage of electrons from the respiratory chain thereby increasing ROS levels in a viscous cycle that over time causes mitochondrial dysfunction and sarcopenia. Early measurements in whole tissue homogenates argued against this hypothesis due to a low <0.1% mtDNA deletion rate [82,83]. While subsequent investigations using quantitative PCR found that mtDNA deletions are not distributed equally throughout all cells but accumulate to high levels in a subset of fibers, suggesting that analyzing whole tissue homogenates would under-represent the single fibers where damage was accumulating [84]. Subsequent analysis using laser capture micro-dissection from sarcopenic rats have shown mtDNA deletions co-localize with electron transport system abnormalities, fiber atrophy, and splitting [80].

Regular vigorous physical activity can attenuate the age-associated decline in mitochondrial function in skeletal muscle in older individuals. A recent cross-sectional analysis of sedentary older individuals in comparison to both active older and young individuals [85] reinforce the results of others that mitochondrial function can be maintained as humans age [16]. Interestingly, the manganese superoxide dismutase (MnSOD) enzyme isolated from sedentary older subjects was found to be highly damaged, as measured by nitration of tyrosine residues which may reduce the function of the enzyme [86]; and suggests that the intracellular environment was accumulating damage to proteins directly related to ameliorating oxidative stress. Those results are further supported by a recent endurance training study on the PolG mouse, which has a defective mtDNA polymerase gamma gene product. The PolG mutator mouse provided the first direct cause-and-effect evidence that mtDNA mutagenesis and mitochondrial dysfunction results in an aging phenotype as it exhibits elevated mtDNA point mutations, mitochondrial dysfunction and accelerated aging. However, five months of endurance exercise attenuated the decline of mtDNA copy number, ETC subunits and complex IV activity (cytochrome c. oxidase) in skeletal muscle, along with the heart, liver, brain, pancreas, and gonadal tissue. [87]. It is clear that vigorous exercise, whether endurance or resistance, has profound protective effects on skeletal muscle and can reverse or slow the well documented declines in mitochondrial function with age in older individuals. However, it is yet to be seen if similar results can be achieved in cases where more severe muscle atrophy and dysfunction are present [39–41].

Concluding remarks and future perspectives

In the present review a decline in mitochondrial function is implicated in the aging process of skeletal muscle. However, many important questions remain unanswered including the physiological significance of declining maximal and basal ATP production rates, and the relationship between protein turnover, quality and function (box 3). It is proposed that many age-related problems in skeletal muscle including the decline in muscle protein synthesis and possibly protein degradation, highly ATP dependent processes, are secondary to reduced MAPR. Further, addressing clinically relevant outcomes together with mitochondrial and muscle function in future research will help us understand how mitochondria are involved in declining muscle function. Only the development of novel methodologies and their application to the study of aging skeletal muscle will provide satisfactory answers. Undoubtedly however, rigorous exercise ameliorates reductions of mitochondrial function in aging skeletal muscle and understanding its effects on skeletal muscle mitochondria have come to the forefront of aging research.

Text Box 3

Impact of aging on skeletal muscle mitochondria

What is the cause of the decline in mitochondrial function with age?

Although studies have clearly showed that both maximal and resting state mitochondrial ATP production rates decline with age the underlying mechanism is still largely elusive. Declining mtDNA copy number is one possible cause of declining function, as it would reduce template availability for transcription. Alternatively, the accumulation of mtDNA mutation and deletion may explain declining mitochondrial function with age. However mitochondrial function depends on both nuclear and mitochondrial DNA and mtDNA encodes only 13 of more than 500 proteins that constitute the mitochondria. Moreover, direct links to declining function of proteins is lacking at this time. Uncovering the changes that occur with age that cause a decline in function is an important area of future research.

What is the relationship between mRNA expression and mitochondrial protein content and function?

Expression levels of genes that encode the mitochondria increase in response to a variety of stimuli such as acute endurance exercise. However how and when those increases are translated into changes in protein synthesis is largely unknown. The protein concentration depends not only on synthesis but also on protein degradation. Moreover, these two processes are critically important to maintain the functional quality of proteins. Further, methodological and technological advances now allow for measuring the synthesis of individual mitochondrial proteins and provide a promising platform to uncover exactly how changes in the expression of genes that encode mitochondrial proteins influence the turnover and functions of those proteins.

What role does exercise training, both resistance and endurance, play in reversing sarcopenia in individuals over 80 years of age?

The specificity of exercise training (endurance vs. strength) on the phenotype of muscle physiology in relatively younger people is well known. However the role that both play in reversing sarcopenia in elderly individuals over 80 years of age remains to be determined. Further, how the muscle selectively responds to a specific exercise program and whether very advanced age modifies the response remains to be explored and likely holds promising therapeutic targets for the sarcopenia of aging.

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Glossary

AMP-activated protein kinase (AMPK)	is the common abbreviation for 5'-adenosine monophosphate activated protein kinase, a serine/threonine protein kinase that is sensitive to the energy status of the cell and generally activates energy producing pathways and inhibits energy consuming pathways
Arteriovenous oxygen difference	the difference in oxygen concentration between the arterial supply and venous return to determine tissue oxygen uptake
Mitochondrial content	is a measure of the amount of mitochondrial protein per volume of tissue. It is commonly measured by the abundance of mitochondrial enzymes, or intact organelles as measured via microscopy
Mitochondrial function	represents the ability for the mitochondria to maintain cellular energetic homeostasis and is commonly expressed as maximal ATP production rate, oxygen consumption (respirometry) or oxidative capacity. It is often normalized to a measure of mitochondrial content
Mitochondrial dysfunction	is a concept that describes an impaired ability of mitochondria to produce ATP or increased ROS production per unit of mitochondria and is measured in the same manner as mitochondrial function, expressed relative to a measure of mitochondrial content
Mitochondrial biogenesis	is the making of new mitochondrial proteins and is measured directly by measuring the rate of synthesis of the proteins that constitute the mitochondrial reticulum, using tracer isotopes (i.e. amino acids or water)
Mitochondrial fusion/fission	two ongoing and dynamic antagonistic processes that separate (fission) or incorporate (fusion) portions of the mitochondrial reticulum
Oxidative capacity	represents the ability of mitochondria to generate ATP and is commonly measured by <i>in vivo</i> by phosphocreatine recovery kinetics (MRS), or <i>in vitro</i> by firefly luciferase or high-resolution respirometry
Peroxisome-proliferator receptor γ co-activator 1α (PGC-1α)	is a transcriptional cofactor of nuclear genes encoding key mitochondrial signaling proteins
Reactive oxygen species (ROS)	are a class of molecules characterized by an unpaired electron on oxygen atoms and are constantly produced in aerobic organisms as

a by-product of oxygen metabolism. ROS play important signaling roles in the cell but can exceed antioxidant capacity and cause oxidative damage to cellular components including proteins, lipids and DNA. The free-radical superoxide ($O_2^{\bullet-}$) is the most common form of ROS and is normally rapidly converted by superoxide dismutase into hydrogen peroxide and oxygen. Subsequently both catalase and glutathione peroxidase can reduce hydrogen peroxide into water thus eliminating any toxic effect

Sarcopenia

is the progressive decline in skeletal muscle mass and strength that occurs with age

Silent mating type information regulation 2 homolog 1 (SIRT1)

is a member of the NAD^+ -dependent protein deacetylase family of enzymes

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TEXT Box 1**Methodological approaches to assessing mitochondrial function**

Multiple measurements are available to investigators to assess mitochondrial function. Investigators may choose a measurement based upon the question they are asking and/or the instrumentation available to them. Common approaches include:

In vitro

- **Firefly luciferase** – Measures the maximal rate that mitochondria can produce ATP through catalyzing the following reactions and measured with a luminometer:
 - Luciferin + ATP → Luciferyl adenylate + PPi
 - Luciferyl adenylate + O₂ → Oxyluciferin + AMP + Light
- **High-resolution respirometry** – Measures the rate of oxygen consumption of isolated mitochondria or permeabilized muscle fiber. An advantage of this approach is the ability to assess the respiratory capacity at multiple levels of the respiratory chain, including uncoupling.
- **Enzyme activities** – Using extremely small amounts of tissue the activities of key enzymes such as citrate synthase, succinate dehydrogenase, or cytochrome c oxidase are measured to assess mitochondrial capacity from frozen tissue. Due to the small sample size the assay is useful in human studies where samples are limited. However extrapolating from one or several enzyme activities to the entire organelle is likely less accurate than other approaches.
- **mtDNA Copy number** – The number of mitochondrial DNA copies per nuclear genome marker (such as ribosomal RNA 28S) is a marker of mitochondrial content [5,16]

In vivo

- **Magnetic resonance spectroscopy (³¹P-MRS)** – ³¹P-MRS is capable of noninvasively measuring maximal ATP synthesis rate in human skeletal muscle, often termed oxidative capacity. A participant is asked to perform a brief bout of muscle activity and the recovery rate of phosphocreatine is measured. The recovery rate constant is proportional to muscle ATP synthesis rate, which is calculated as the product of the recovery rate and concentration of phosphocreatine in the resting skeletal muscle.
- **Maximal oxygen consumption (VO_{2max})** – VO_{2max} is measured at the whole-body level and is approximated by indirect calorimetry during an exercise test. While VO_{2max} is dependent upon the body's mitochondria for the extraction of oxygen, it is also a function of the oxygen-carrying capacity of the blood, tissue perfusion and cardiac output making it an integrated measurement of metabolism.

Together the *in vitro* approaches provide mechanistic information that cannot be accessed by *in vivo* approaches, however, the physiological relevance is often questioned. *In vivo* approaches are non-invasive and physiological relevant, however mechanistic information is not possible along with the assumptions, and mathematical modeling is often questioned.

Text box 2**Mitochondrial fusion/fission – an emerging area of importance in mitochondrial aging**

Mitochondria exist as a dynamic reticulum that continuously exchange components during fusion and fission events [102] that can be altered with aging. Mitochondrial fusion and fission help maintain mitochondrial function by exchanging components such as membrane, proteins, and DNA. However, it is not likely that fusion events can repair the membrane because fusion requires an intact membrane potential (which can dissipate with damage). Instead, fission of the membrane for transport to the lysosome can occur following loss of membrane potential and allows damaged portions of the reticulum to be degraded by autophagy [34]. The loss of the mitochondrial inner membrane fusion protein Mgm1P resulted in fragmented mitochondria and decreased lifespan in *S. cerevisiae* mutants compared to wild-type [103]. Mice with impaired mitochondrial fission developed skeletal muscle atrophy and show that altered mitochondrial dynamics can lead to sarcopenia [104]. Thus, a loss of fission proteins with aging could decrease the removal of damaged proteins and contribute to an accumulation of damaged mitochondria.

Figure 1. Mitochondrial protein turnover

The turnover of mitochondrial DNA (mtDNA) encoded proteins may occur independently of nuclear DNA (nDNA) encoded proteins and can be regulated in part by damage to mitochondrial proteins. Moderately damaged mitochondrial proteins can be degraded by intrinsic proteases (Lon) while severely damaged proteins can dissipate the membrane potential (ψ) and lead to fission of the membrane for degradation by the lysosome (mitophagy). The free pool of amino acids in the mitochondria reflects the amino acid content of mitochondrial proteins encoded by mtDNA [101] and suggests the mitochondrial free amino acid pool is sustained by degradation of mitochondrial proteins. AMPK and downstream targets help coordinate the expression of nDNA and mtDNA encoded proteins. Abbreviations: AA [Amino acids], AMPK [AMP activated protein kinase], LON [Lon protease], mTOR [Mammalian target of rapamycin complex 1], PGC-1 α [Peroxisome proliferator-activated receptor γ coactivator 1 α], ROS [Reactive oxygen species], SIRT1 [sirtuin 1].

Figure 2. Age associated changes in muscle mitochondrial function

Damaged skeletal muscle proteins with less function are normally degraded and replaced with newly synthesized proteins. Aging is associated with the accumulation of damaged proteins through both increased rates of damage and decreased degradation.

Figure 3. The role of declining mtDNA copy number on skeletal muscle aging

A hypothetical model that shows how declining mtDNA copy number in aging skeletal muscle may relate to sarcopenia, and the role of exercise training on reversing sarcopenia.

Table 1

Age effect on mitochondrial ATP production

A comparison of studies on the oxidative capacity of skeletal muscle and aging organized by measurement type and year of publication.

Ex Vivo Studies	PA Matched	Muscle	Preparation	Normalization	Results	REFS
Enzyme activity and respirometry	No	VL	HP IM	TWW MP	Age effect	[88]
Enzyme activity and respirometry	No	VL	IM IM	MP MP	Age effect	[89]
Enzyme activity	Yes	VL	HP	TWW	Age effect	[90]
Respirometry	Yes	VL	IM	MP	No age effect	[91]
Respirometry Enzyme activity	Yes	VL	IM HP	MP HP	No age effect	[92]
Enzyme activity	Yes	VL	IM & HP	MP & HP	Age effect	[12]
Enzyme activity	Yes	VL	HP	TWW	Age effect	[23]
Respirometry	Yes ^a	VL	IM	MP	No age effect	[19,2 7,85, 93,94]
Enzyme activity and respirometry	Yes	VL	IM PF & IM	MP CS & IM	Age effect	[13]
Enzyme activity and respirometry	Yes	VL	HP IM	TWW MP	No age effect	[75]
Enzyme activity and maximal ATP	Yes	VL	IM	MP	Age effect	[5]
Enzyme activity and respirometry	No	VL	PF	TWW & CS	No age effect	[95] ^b
Maximal ATP production rate	Yes	VL	IM	MP	Age effect	[16]
In Vivo Studies	PA Matched		Measurement		Results	
Maximal ATP flux	No	Gastroc &	MRS		Age effect	[14]
Enzyme activity	Yes	Deltoid	HP	TWW	No age effect	[96]
Maximal ATP flux	Yes	VL	MRS		Age effect	[17]
Maximal ATP flux	Yes	TA	MRS		No age effect	[97]
Basal ATP flux	Yes	Soleus	MRS		Age effect	[18]
Maximal ATP flux	Yes		MRS		No age effect	[98]
Maximal ATP flux	Yes	TA	MRS		No age effect	[99]
Basal ATP flux	No	TA & FDI	MRS		No age effect	[100]
Maximal ATP flux	Yes	VL	MRS		Age effect	[24] ^c

PA, physical activity.

^aIndicates the study controlled for PA but unlike the other studies that included only one type of participant, such as sedentary, or recreationally active, the study of Rasmussen et al included a range of PA levels and matched across young and older participants.

^b Measured in permeabilized muscle fibers.

^c Measured in both the VL and TA with an age effect found in the VL but not the TA.

Abbreviations: tissue wet weight (TWW), mitochondrial protein (MP), citrate synthase (CS), isolated mitochondria (IM), homogenate protein (HP).