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Aging induced structural alterations in SR-Mitochondria interaction in skeletal muscle: Emerging insights

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eLife Assessment

The Review Article by Bal and co-workers presents an overview of skeletal muscle physiology, focusing on Sarcoplasmic Reticulum and Mitochondria-Associated Membranes (MAMs) in relation to calcium handling, ROS, and signaling. It provides a foundation based on the current literature but could have gone further by identifying future research directions and potential avenues for therapeutic intervention.

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Abstract

Skeletal muscle undergo remarkable changes during aging including anatomical, ultrastructural, and moreover biochemical. The aging associated reduction of muscle mass, termed as sarcopenia, is a major factor in geriatric functional decline and frailty, contributing to the lowering of self-confidence. In an adult skeletal muscle fibers, sarcoplasmic reticulum (SR) and mitochondria exhibit most intricate and precise distribution along with the sarcolemmal (forming T-tubule), which is critical for muscle function. In healthy young muscle tissue, the close physical proximity of SR and mitochondrial membranes shows contacts called mitochondria-associated membranes (MAMs). Recent literature highlights the role of MAMs network in smooth functioning of muscle by regulating localization of Ca2+-signaling, lipid transport, and other signalling molecules like reactive oxygen species. Several tethering mechanisms are proposed to stabilize the MAMs network, the classical ones being the mitofusins (MFN1 and MFN2). Emerging consensus suggest that MAMs in the skeletal muscle facilitate accuracy of excitation-metabolic coupling ensuring spatial energy supply. However, upon aging the precision of SR and mitochondria co-localization as well as crosstalk seems to be affected. In this review, we have critically examined the current literature about MAMs network structure and function during health and diseases mainly from an aging perspective. We have further evaluated the role of exercise, nutritional, nutraceutical and pharmacological approaches in lowering MAMs loss in an effort to retard aging progression. Retention of skeletal muscle health and performance is a major factor in achieving the goal of healthy aging.

1. Introduction

Skeletal muscle is far more than a simple mechanical machine for locomotion with several homeostatic functions [1]. It is a major organ contributing more than 40 percent of body mass and energy utilization; hence a focal point of metabolism [2]. However, muscle mass starts to decline with aging process compromising physical performance that is generally known as “Sarcopenia” [3, 4]. Interestingly, the whole-body metabolism is also regulated indirectly by skeletal muscle via serving as a source of myokines [5, 6]. In terms of biochemical mechanisms of skeletal muscle, calcium (Ca2+) plays a vital role both in governing the contractile performance as well as regulating metabolic adaptability [7, 8]. In the skeletal muscle the two key organelles namely sarcoplasmic reticulum (SR) and mitochondria regulate the myoplasmic Ca2+-dynamics [9]. The SR is the major Ca2+-store inside the myocyte and is very intricately organised forming junctions with

sarcolemma (on T-tubule) that are spatially localized on the sarcomere [10]. Emerging data highlight mitochondria as an alternative Ca2+-store and also show very precise spatial arrangements across the sarcomere with junctional contact points with the SR [11, 12]. In addition, skeletal muscle exhibit remarkable diversity in their functions, metabolic profiles, and structural characteristics depending on the metabolic requirements [13, 14]. Individual skeletal muscle fibers are categorized into type I (Slow Twitch) or type II (Fast Twitch), based on the myosin heavy chain isoforms [15]. Usually, type I fibers rely on oxidative metabolism and are resistant to fatigue, whereas type II fibers generate rapid, forceful contractions but fatigue quickly [16]. In larger mammals like humans, most skeletal muscles have both the fiber types but in varying ratios. During aging, type II fiber size and number is selectively reduced shifting the overall fiber-type composition toward type I leading to compromization of movement speed [17].

Recent studies show that aging triggers some specific biochemical changes in skeletal muscle that may be considered as the hallmarks of aging [18]. Some of these aging hallmarks are disruption of the Ca2+ homeostasis that include both SR and mitochondria [19, 20]. One of the most prominent factors in the SR is increased leakiness of ryanodine receptor (RyR1) that can originate from faulty post translational modification like oxidation and nitrosylation [21, 22]. The other SR alteration is downregulation in the expression of SERCA pump and aberration in its micropeptide regulators [23]. Apart from biochemical, several structural features reported to undergo changes are deterioration of T-tubule integrity and dyad/triad arrangement and distribution that impair force production and muscle relaxation [24]. Another crucial factor in muscle aging often under-recognized is reduction in metabolic flexibility; which is defined as the ability to rapidly switch fuel sources (glucose and fat) to meet energy demands [25, 26]. Since metabolic flexibility depends on coordinated Ca2+ signals between SR-mitochondria its loss marks a turning point in the progression of sarcopenia. In addition to SR, muscle mitochondria also exhibit several changes during age-related muscle wasting. Such changes include fragmentation and accumulation of damaged mitochondria which impair ATP output affecting metabolic flexibility [27]. Also, aged skeletal muscle mitochondria exhibit several structural changes including cristae arrangements/abundance, intermembranous space [28, 29]. Further, the interplay between SR-mitochondria is dampened due to impairment in tethering (mediated by mitofusins) and MAM (mitochondria-associated membrane) integrity [30]. These changes not only affect intra-myoplasmic Ca2+-homeostasis but also modulate lipid trafficking, and redox balance: three factors collectively drive sarcopenic remodelling [31, 32]. Research over last few decades has shown that neuromuscular denervation and its associated organellar decay also greatly contribute to the progression rate of sarcopenia [33]. These age-related changes create a self-reinforcing cycle: Ca2+ dysregulation amplifies mitochondrial stress that produce reactive oxygen species (ROS), elevated ROS further oxidizes SR channels, and lipid accumulation disrupts physical contacts collectively converging to impaired SR-mitochondrial crosstalk.

In this review, we aim to investigate if the disruption of SR-mitochondrial coupling contributes to the onset and progression of sarcopenia. We particularly analyze the role of the molecular determinants that govern Ca2+-signaling microdomains and the integrity of SR-mitochondrial communication networks. Some recent studies have highlighted that during metabolic diseases; SR-mitochondrial physical proximity is weakened that distorts the localized Ca2+ fluxes causing asynchrony in muscle function including ATP production and SR Ca2+-cycling [31].

In addition, we also examine how the impairment in SR-mitochondrial tethering, Ca2 +- transport machinery and redox balance collectively drive the pathogenesis of sarcopenia in an effort to understand the molecular mechanism. Insight into these molecular interdependencies may ultimately reveal novel therapeutic targets for preserving muscle mass, strength, and metabolic flexibility in aging populations enabling “healthy aging”.

2. SR as the Primary Regulator of Ca2+ Homeostasis

In the skeletal muscle, SR is a highly branched network of membranes that is intricately associated with the muscle myofibrils [34]. The terminal cisternae of SR are positioned around T-tubules to form triads, which are the sites of excitation-contraction (EC) coupling [35]. This organization

provides for both rapid and efficient coupling of Ca2+ release to the contractile machinery. EC-coupling relies on a physical interaction between dihydropyridine receptor (DHPR; contain a voltage sensors, Cav1.1) on T-tubules and Ca2+-release unit (CRU) on the SR membrane. Each cluster of DHPR aligns with alternating tetramers of RyR1 in the CRU, a typical pattern seen in the electron microscopy. When depolarization occurs, the DHPR undergoes a conformational change that mechanically opens the RyR1, leading to the swift release of Ca2+. Junctophilin 1 and 2 create a 10-15 nm distance between the SR and the T-tubule to allow tight voltage-Ca2+ coupling. The triad structure is stabilized due to junctional proteins JP-45 and mitsugumin 29 that also modulate RyR1 sensitivity [36]. The SR Ca2+- storage is facilitated by Ca2+ binding proteins like calsequestrin 1 (CASQ1), S100, calceneurin, calreticulin [37, 38]. CASQ1 is the major Ca2+-buffering protein that can undergo reversible polymerization in a Ca2+-dependent manner. CASQ1 interact with the RyR1 mainly through triadin and junctin (direct interaction also has been reported), thereby keeps high amount of Ca2+ close to the CRU [39]. Alteration in interactions among these proteins has been reported in several muscle diseases like malignant hyperthermia, vacuolar myopathies [40, 41].

After the Ca2+-release from SR to the myoplasm, SERCA pumps dominate Ca2+-reuptake back into the SR. While, SERCA1a in fast-twitch muscle fibers, SERCA2a predominate in slow-twitch muscle fibers and heart that caters to specific contractile demands of each muscle types [42]. In addition to ATP availability, SERCA activity is regulated by several mechanisms such as post-translation modifications (phosphorylation, acetylation, nitrosylation, sumoylation, glutathionylation) [43]and micropeptide regulators like sarcolipin (SLN) and phospholamban (PLB) [44]. The rate of Ca2+-reuptake is further determined by the extent of protein expression of SERCA isoforms, SLN and PLB. The SERCA Ca2+-removal is also regulated by proteins like protein kinase A (PKA) or Ca2+/calmodulin-dependent kinase II (CamKII) that influence SLN and PLB activity [45]. Interestingly, based on SERCA activity local Ca2+-signals can be generated in the myoplasm affecting nuclear transcription as well as mitochondrial Ca2+-flux. Aging interferes with both phases of Ca2+ homeostasis as a decrease in junctophilin expression increases the SR and T-tubule gap, delaying Ca2+ release and decreasing peak tension. Also, decreased expression of SERCA isoforms with an increase in slower isoforms has been shown during aging leading to decreased relaxation and contractile efficiency [46]. Though secondary Ca2+ clearance processes, such as plasma membrane Ca2+ ATPases (PMCA) and sodium-Ca2+ exchangers (NCX), can provide compensatory capacity when SR reuptake is impaired, these mechanisms cannot match the efficiency of SERCA for maintaining Ca2+ homeostasis.

Studies in last few decades have shown that Ca2+-flux into the mitochondria is usually influenced by its proximity to the SR and MAM density [46]. MAMs are established when the SR and mitochondria membranes are closely juxtaposed at 10-30 nm range and tethered together by protein complexes especially mitofusins (MFN1 and MFN2) [47] (Figure 1 ↗). Other protein complexes that assist MAM formation are voltage-dependent anion channel 1 (VDAC1), glucose-regulated protein 75 (GRP75), and inositol 1,4,5-trisphosphate receptor (IP3R) in addition to PACS-2(Phosphofurin acidic cluster sorting protein 2) [48]. It is considered that MAMs facilitate efficient Ca2+ transfer and metabolic coupling to tune the muscle's energy demands. The structural arrangement of the MAMs is found to be affected in various physio-metabolic conditions. Exercise (especially aerobic) mediated skeletal muscle remodelling improves MAM function thereby reducing insulin resistance. In contrast, both sarcopenia and type 2 diabetes have been reported to disruption the MAM structure in skeletal muscle [49]. Hence, many researchers have proposed use of MAM abundance an early biomarker for sarcopenia [50].

In the skeletal muscle, SR Ca2+-homeostasis is also a critical regulator of the neuromuscular junction (NMJ) activity which in turn affects the recruitment of muscle contractile machinery [51]. The subsynaptic SR Ca2+ transients stringently govern level of acetylcholine receptor and other synaptic proteins mainly through the agrin-Muscle-Specific Kinase (MuSK)-GA-binding protein (GABP)/N-box signaling axis and cooperatively through signaling pathways including calcineurin and nuclear factor of activated T-cells (NFAT), and are further modulated by neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor

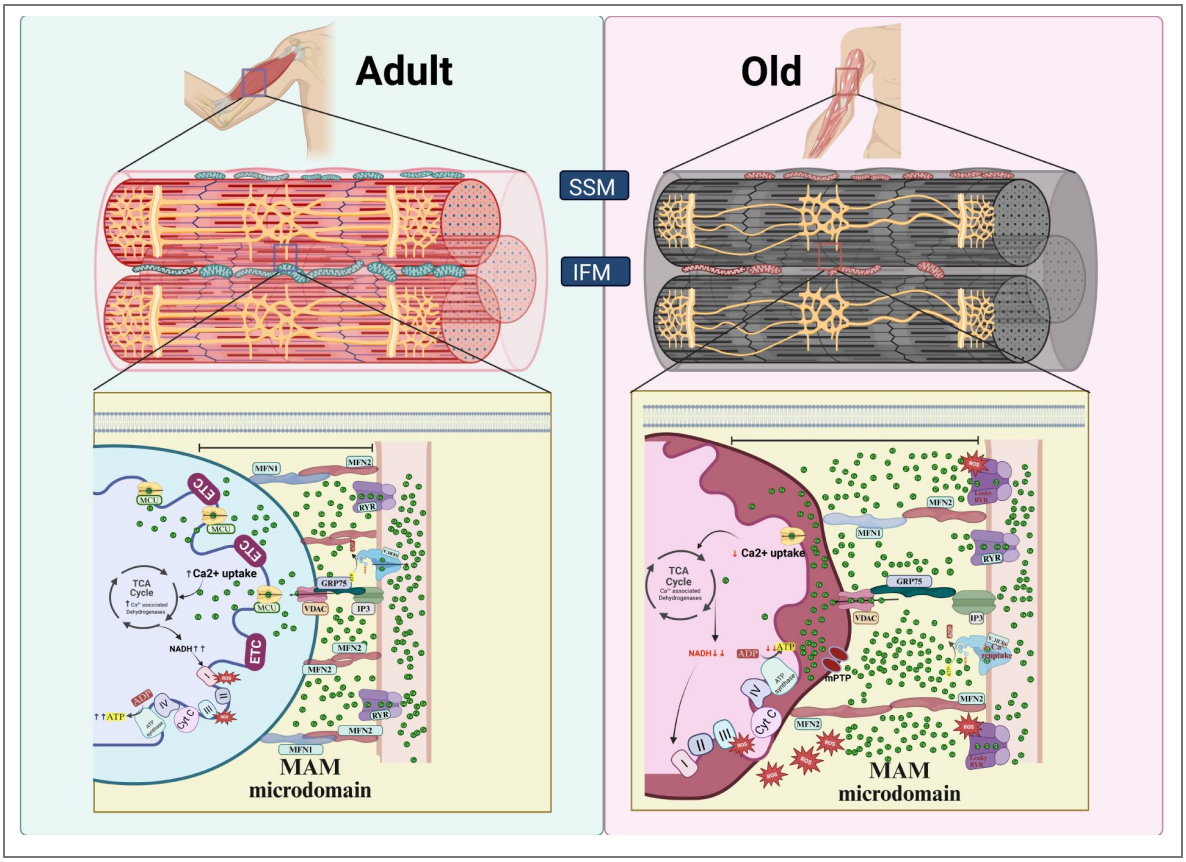

Figure 1. Structural arrangement of the skeletal muscle undergoes remarkable changes in old compared to adult at all levels of organization.

The top panel shows skeletal muscle arrangement in old (right, pink background) versus adult (left, blue background). The middle panels compare the myofiber cross-sections from adult and old muscles. In adult muscle, the SSM and IFM populations are abundant and well-organized, whereas in old muscle their abundance is markedly reduced (especially IFM) and their (ultra)structural features are impaired. The lower panels compare molecular organization of MAM in adult versus old muscle. In adult muscle, MFN1/2, GRP75, VDAC, and IP_3R hold the SR and mitochondria in close proximity. Ca2+ released through RyR1 builds up locally termed "Ca2+-microdomain", which ensure quick mitochondrial Ca2+-influx that activate TCA dehydrogenases thereby enhancing NADH and ATP output and the ETC runs without significant ROS leak. In old muscle, the gap between SR and mitochondria widens as expression of MFN2 and GRP75 decline without a defined Ca2+-microdomain. The MCU uptake slows limiting the mitochondrial Ca2+-influx that reduce ATP production and sustained SERCA activity. In addition RyR1 becomes leaky mainly by ROS-mediated posttranslational modifications leading to increased cytosolic Ca2+ levels. This figure was created using *BioRender.com*.

(GDNF) [52, 53]. While synaptic SR arrangement is derailed by pathological conditions including aging, physical exercise and high-frequency electrical stimulation restores the synaptic SR structures and its Ca2+ signaling [54]. In essence, the local Ca2+ signaling produced by the SR plays an important role in mediating both contraction and connectivity of the neuromuscular unit [9]. Furthermore, the SR Ca2+- homeostasis critically govern muscle mitochondrial abundance by modulating mitochondrial dynamics (fission/fusion), and rate of mitophagy in which MAMs may be considered to be integral component [55].

3. Mitochondria: A factor in muscle aging

In muscle, mitochondria plays the role of a dynamic player; not only producing 90% of ATP used but also acting as a buffer for Ca2+ and regulating redox signalling [56]. Mitochondria occupy more than 10% of the muscle volume and adapt their ATP output to the fluctuations of contractile demand and Ca2+ level. Spatially in myofibers [56], mitochondria are organized into subsarcolemmal populations (termed SSM) beneath the cell membrane that support energy demand of ion balance, and intermyofibrillar populations (termed IFM) nestled between myofibrils fueling contraction, also bear greater capacity of Ca2+-buffering [57, 58]. Mitochondria take up Ca2+ via the mitochondrial Ca2+ uniporter (MCU) complex regulated by mitochondrial Ca2+ uptake (MICU) 1, MICU2, MICU3, and essential MCU regulator (EMRE) [59, 60]. In muscles, MAM is proposed to form specialized microdomains that can amplify the rate of mitochondrial Ca2+-influx enhancing NADH generation and ETC activity; thereby facilitating EC-metabolism coupling ensuring adequate ATP supply [61]. Studies involving deletion of MCU or MICU has shown that both excess and insufficient mitochondrial Ca2+ uptake can compromise muscle function. Excess Ca2+ leads to fiber type shift toward glycolytic and impairs endurance; while inadequate Ca2+, disrupts energy homeostasis and reduces performance [13, 62]. In skeletal muscle, mitochondrial function is also influenced by ROS and reactive nitrogen species (RNS). Mitochondrial phenotype is also modulated by dynamics, mitophagy, biogenesis and ultrastructure (cristae abundance). Mitochondria generate ROS as byproducts of electron transport, primarily at complexes I and III [63]; that is proposed to be essential for exercise-induced mitochondrial biogenesis. ROS, at physiological concentrations, can activate NRF2-mediated antioxidant gene expression, MAPK (Mitogen-Activated Protein Kinase) cascades, and Ca2+-dependent kinases [63]. RNS, including nitric oxide and peroxynitrite, similarly regulate vasodilation and intramuscular substrate supply, which is closely intertwined with mitochondrial dynamics and muscle plasticity [64].

Muscle mitochondrial network undergoes regular fission and fusion to maintain a functionally active structure and selectively remove damaged parts (Figure 2). Fusion proteins like MFN1, MFN2 and OPA1 (Optic Atrophy 1) facilitate elongation of mitochondrial network, whereas fission proteins like DRP1 (Dynamin-related protein 1), MFF (Mitochondrial Fission Factor), FIS1 (Mitochondrial Fission 1), MIDs (mitochondrial dynamics proteins) facilitate division thereby restricting the network [29]. Mostly, fission selectively removes dysfunctional mitochondrial portions that lead to mitophagy mediated by proteins, including PINK (PTEN-induced putative kinase) and Parkin [65]. Being the site of ETC machinery, inner mitochondrial membrane architecture is crucial for sustaining high intracellular respiratory status. So, high metabolic demand (like cold, exercise) remodels cristae organization that is facilitated by proteins of mitochondrial contact site and cristae organizing system (MICOS) [66, 67]. Above all, mitochondrial plasticity is dependent on its biogenesis, which requires synergistic expression of nuclear and mitochondrial genes. In skeletal muscle, PGC-1a (Peroxisome proliferator-activated receptor [PPAR] gamma coactivator 1-alpha) is often labeled as “master regulator” of mitochondrial biogenesis [68]. PGC-1a, along with nuclear respiratory factors (NRF1 and NRF2), PPAR, and ERRa, modulate nuclear genes needed for mitochondrial biogenesis. TFAM (mitochondrial transcription factor A), on the other hand, is key for the regulation of mitochondrial DNA (mtDNA) replication and transcription [69]. Interestingly, contractile activity, especially through Ca2+ and ROS signalling mediate mitochondrial biogenesis by inducing PGC-1a through AMPK, p38 MAPK, and calcineurin pathways, leading to an oxidative phenotype [64].

However, mitochondrial ROS accumulation (during pathophysiological conditions) causes oxidative damage to proteins and mtDNA, affecting energy turnover. Oxidative modifications to ETC proteins reduce their electron transfer efficiency, creating a vicious cycle of increased ROS production, damaging not only mitochondria but also SR Ca2+-handling proteins, including RyR1 and SERCA [70]. These myocellular processes undergo progressive changes during aging, which are detailed in the following sections.

Aging in skeletal muscle not only reduces performance but also leads to several changes in mitochondrial properties. A remarkable change is a reduction in mitochondrial abundance as well as expression of MCU and MICU3, which impedes the efficiency of Ca2+-stimulated respiratory capacity [62, 71]. Next, a major change with aging is a shift in balance toward excessive fission and impaired mitophagy due to increased DRP1 activity, while fusion proteins and autophagy machinery decline. This results in the accumulation of fragmented mitochondria that produce more ROS and harbor mtDNA deletions, confirmed by proteomics and genomics studies of aged muscle [72, 73]. Interestingly, the fall in mitochondrial abundance in aged skeletal muscle is proposed mainly due to a reduction in biogenesis capacity. Aging impairs muscle plasticity as $PGCl_{\alpha}$ induction by exercise is blunted, which slows mitochondrial biogenesis, jeopardizing oxidative capacity [74]. Obviously, suppression of $PGCl_{\alpha}$ expression decreases mitochondrial content, shifting muscle metabolism toward glycolysis, which is an analogous phenomenon in the case of disuse or denervation. Aging particularly reduces IFM function, impairing location-specific ATP supply for contraction, contributing to weakness [58]. Further, age-related disruption of MICOS components gives rise to cristae disorganization, impaired oxidative phosphorylation, and altered susceptibility to apoptosis [67, 75]. Aging muscle exhibits reduced antioxidant capacity alongside increased mitochondrial ROS production, disrupting redox homeostasis [76]. In severe cases of aging, as in sarcopenia, mitochondrial damage leads to opening of the permeability transition pore (mPTP) and loss of membrane potential, which has been suggested as a reason for fiber injury [77]. In aging muscle, Ca2+ and ROS signaling in mitochondria undergo significant remodeling, and the degree of these changes may determine aging progression, implicating altered mitochondria-SR interplay as a central contributor [72, 77].

4. SR-Mitochondrial Communication Networks

Ultrastructural studies, including TEM and 3-D tomography, have shown that apart from the T-tubular structure, the SR and mitochondrial membranes form a major portion of the intramyocellular membrane network in skeletal muscle. It is increasingly accepted that SR and mitochondria align dynamically within 10-30 nm upon exposure to metabolically demanding conditions. These structures are often termed MAMs and are proposed to form microdomains of intricate interaction between the two organelles. Recent studies have identified several proteins that stabilize these microdomain structures and suggest their role in orchestrating Ca2+ and lipid exchange, influencing muscle metabolism(Figure 3).

Structural arrangement and tethering

Structural data show that mitochondrial outer membrane is physically juxtaposed against the SR at MAMs contact points held in place by tethering proteins; the primary ones being the MFNs. While MFN1 is exclusively localized on mitochondria, MFN2 is expressed on both the organellar membranes [78, 79]. Some studies also suggest MAMs formation by interaction between IP_3R on the SR to VDAC1 on mitochondria through a bridge by GRP75 [80]. In skeletal muscle, approximately 5-20% of the outer mitochondrial membrane bear MAMs that enables rapid, localized Ca2+ transfer without substantially elevating bulk cytosolic Ca2+ levels. Within these junctions, Ca2+-released through RyR1 and IP3Rs generates high-Ca2+ microdomains (>15 μM) sensed by mitochondria via the IP3R-Grp75-VDAC-MCU axis [81], stimulating ATP production. Importantly, the stability of MAMs is further enhanced by anchoring of SR to T-tubules at the triads by proteins like junctophilins (JPH) [82]. TEM reveals electron-dense MAMs contact points are abundant in oxidative muscle fibers where energy demands are high [49]. Interestingly, these proteins exhibit variation in their expression in fast-twitch and slow-twitch skeletal muscle fibers.

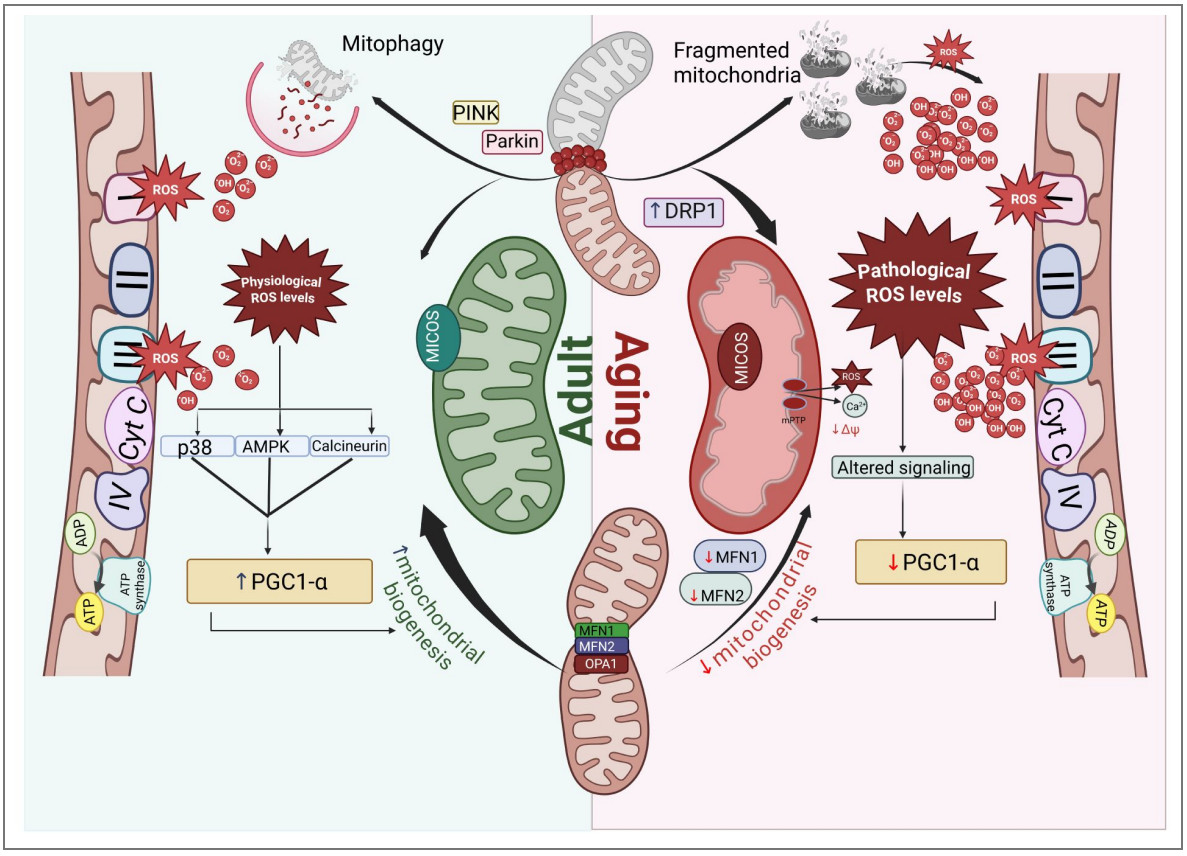

Figure 2. Comparison of molecular determinants of mitostasis in skeletal muscle of adult versus old.

Mitostasis is defined by the rates of mitochondrial biogenesis (determined by PGC-1a), dynamics (determined by fusion vs. fission) and mitophagy (determined by PINK/Parkin). In adult muscle (left in blue background), mitochondrial biogenesis is optimal due to ROS from the ETC activates p38, AMPK, and calcineurin, converging on PGC-1a. MFN1/2 and OPA1 sustain favourable mitochondrial network, and PINK/Parkin clears damaged mitochondria before they accumulate maximizing efficient mitochondrial abundance. The proteins responsible for cristae structure maintenance (like MICOS complex) are expressed abundantly that sustain well-organized cristae quantity. Contrastingly, in aging (right in pink background) ROS-level rises to pathological levels, DRP1 activity increases, and the mitochondrial network becomes more fragmented. Expression of MFN1 and MFN2 fall leading to lower fusion rate along with loosening of MAM tethering. MICOS complex gets disorganized leading to deformed cristae and compromised oxidative phosphorylation. Both mitophagy and biogenesis are lessened leading to accumulation of dysfunctional mitochondria. This figure was created using *BioRender.com*.

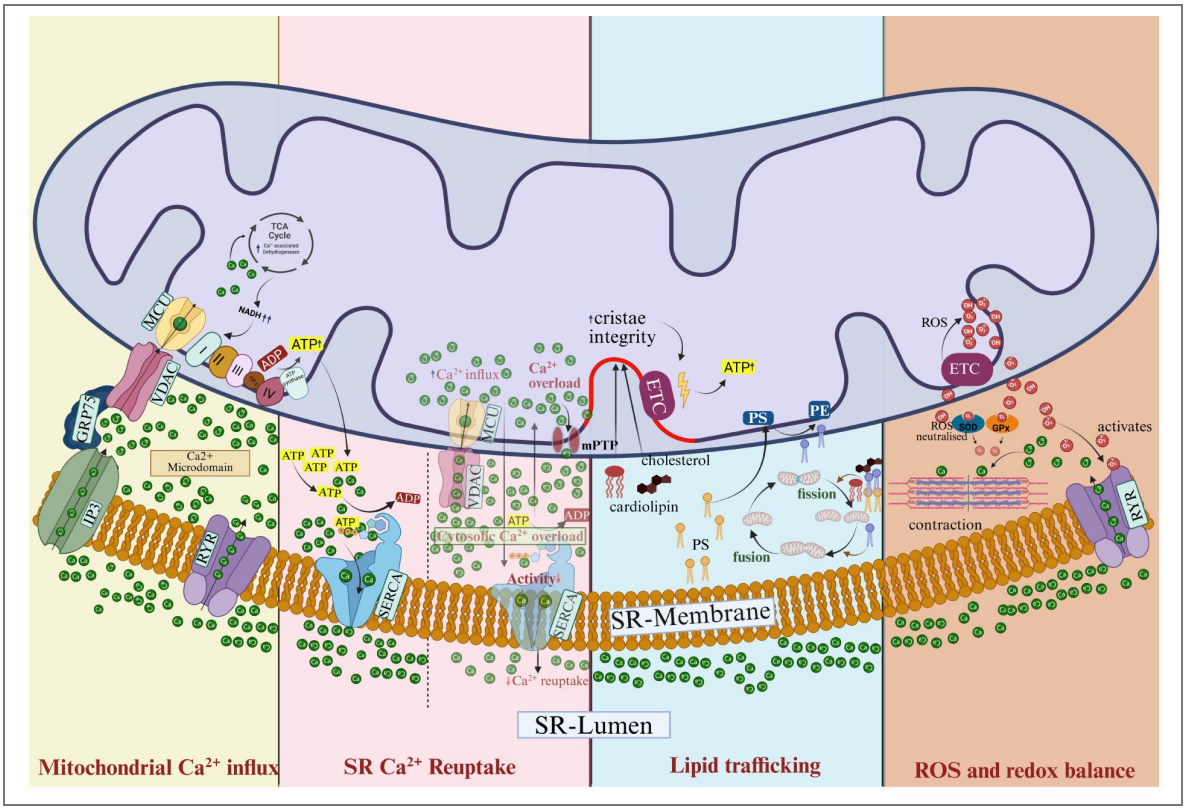

Figure 3. Various physiological functions of MAMs under normal conditions.

This schematic presents the typical architecture and function of MAMs in healthy skeletal muscle. The four panels depict mitochondrial Ca2+ influx, SR Ca2+ reuptake, lipid trafficking, and ROS/redox balance that play an integral role in physiological conditions. In the adult muscle, the MAMs integrity ensures efficient mitochondrial Ca2+ influx to facilitate aerobic metabolism and ATP supply. The panel on "SR Ca2+ reuptake" highlights SERCA function in Ca2+ transport back into the SR lumen during normal and cytosolic Ca2+-overload during abnormal conditions. MAMs-mediated lipid trafficking plays a critical role in sustaining relative composition of various lipids and cristae organization supporting mitochondrial dynamics and abundance. ROS generation is maintained at physiological levels, with local antioxidant enzymes (SOD, GPx) preserving redox-sensitive signalling. The structural integrity of the SR membrane and lumen is depicted, highlighting the coordinated regulation of Ca2+, energy, lipid, and redox homeostasis that defines healthy excitation-metabolism coupling. This figure was created using *BioRender.com*.

While, MAMs structure was shown to be higher in slow-twitch over fast-twitch fibers; the abundance of MFN1/2 has been debated. Expression of MFN1 is suggested to be higher in slow-twitch fibers; but, counter-intuitive expression of MFN2 has been reported. A study on human muscle showed higher expression of MFN2 in fast-twitch fibers, but another study reported MFN2 expression being more responsive to use/disuse in oxidative muscles [83]. This might indicate MFN2's role in more functions than tethering alone as it is a SR-membrane protein. Among alternate tethers, IP3R is expressed more in fast-twitch avian muscles, whereas VDAC1 and GRP75 are usually expressed higher in slow-twitch fibers [84]. Currently, there is no clear data in expression of JPH in a fiber-type specific manner; although it has been suggested JPH1 is preferentially expressed in fast-twitch fibers and JPH2 in slow-twitch fibers [85, 86]. Intriguingly, MAM density declines during postnatal development, as mitochondria remodel to wrap tightly around sarcomeres, indicating that contact site architecture is regulated developmentally and in fiber-type manner [87].

MAMs and Ca2+-influx

Quick Ca2+-flux into the mitochondria is the major function of MAMs by creating Ca2+-microdomains, where local concentrations reach 10-100 μM that is 10 times the myoplasmic Ca2+-concentration during contaction [88, 89]. This bypasses the diffusion delays enhancing inter-organellar communication and boosting mitochondrial ATP production [61, 89]. MAMs allow mitochondria to buffer excess Ca2+ during prolonged or repetitive contractions, preventing SR Ca2+-overload and protecting against excitotoxicity. Obviously, role of MAMs becomes very important during exercise and it has been shown that training enhances expression of MAMs-associated Ca2+-channels including IP3R, RyR1, MCU [90]. Emerging data suggest that exercise-mediated mitochondrial health benefits are partly due to improved MAMs structure and mitochondrial Ca2+ homeostasis [49, 90]. Lowered post-exercise recovery is suggested due to reduced MAMs that causes metabolic inflexibility [25]. Further, all conditions that exhibit deteriorated muscle health including aging, disuse, or metabolic disease have impaired mitochondrial Ca2+ flux and reduced ATP output [31, 61]; raising question of how MAMs architecture is altered. During aging the MAM gaps widen along with decline in expression of MFN2, IP3R and MCU, which slows mitochondrial Ca2+ uptake, resulting in metabolic lag and cytosolic Ca2+ overload [50, 61]. These conditions in aged fibers, correlate with decreased fatigue resistance and reduced capacity for sustained or repeated contractions. Atrophy models like disuse, microgravity and aging exhibit progressively decrease MFN2 levels causing loss of MAMs [91]. On the other hand MFN2 overexpression studies showed partial restoration of MAMs structure improving mitochondrial function in aged muscle [92]. An alternate idea has also been put forward that excessive MAMs density may prolong mitochondrial Ca2+-loading as mitochondrial Ca2+- efflux mechanism (through mNCX) is very slow leading to mitochondrial swelling and apoptosis [93]. So, a balance between MAMs abundance and mitochondrial Ca2+-loading has to be maintained.

MAMs and lipid trafficking

MAMs coordinate multiple aspects of muscle bioenergetics and membrane homeostasis that extend well beyond Ca2+ transfer. MAMs serve as hubs for lipid synthesis and exchange: phosphatidylserine move from SR to mitochondria, where phosphatidylserine is converted to phosphatidylethanolamine. Further, MAMs facilitate cardiolipin and cholesterol delivery to mitochondrial membranes [94, 95]. These lipid trafficking are critical for mitochondrial inner membrane integrity and support mitochondrial biogenesis. This lipid trafficking is complemented by recruitment of mitochondrial dynamics regulators like MFN1/2, OPA1, and DRP1 to MAM sites, enabling fission and fusion remodeling in response to metabolic demands. So, MAM-mediated lipid transfer partly regulates ETC function and reduced contact sites impair this exchange, exacerbating mitochondrial membrane dysfunction and diminishing respiratory capacity [72]. Concurrent lipid accumulation and membrane fluidification defects further impair both Ca2+ transfer and respiratory function, driving a metabolic shift toward anaerobic glycolysis that reflects disrupted SR-mitochondrial dialogue. Interestingly, some studies have shown MAMs

composition and its role in lipid trafficking varies according to mitochondrial subpopulations; subsarcolemmal (SSM) vs. Intermyofibrillar (IFM). SSM near capillaries are particularly enriched in cristae abundance and may serve as rapid energy provider during high-intensity work requiring quick lipid uptake that is facilitated by their related MAMs [57, 58]. In contrast, lipid buildup near failing MAMs is driven by impaired mitochondrial fatty acid oxidation and insulin resistance, which physically disrupts these contacts and further inhibits proper Ca2+ and redox signaling [49].

MAMs and SR-Ca2+ reuptake

Prolonged high Ca2+-concentration in MAMs-microdomains may cause mitochondrial Ca2+-overload leading to dysfunction and that is why Ca2+-reuptake back into the SR is equally important to manage the downside of MAMs. At the same time, MAMs-mediated boosting of mitochondrial metabolism is critical for ATP supply to SERCA-driven Ca2+ reuptake. This energetic feedback loop is very important as SERCA activity accounts upto 90% of resequestertion of Ca2+ into the SR, effecting termination of contraction [46]. So, altered MAM composition reduces mitochondrial ATP-generating capacity that can constrain SERCA activity, slowing Ca2+-reuptake and promoting cytosolic Ca2+-accumulation [70]. Experimental interventions that enhance SERCA function (e.g., CDN1163 or SERCA1 overexpression) improve mitochondrial bioenergetics, probably via stabilizing MAMs and SR Ca2+-reuptake [70]. Also, moderate endurance exercise has been shown to improve MAM efficiency, the SR-mitochondria energy feedback loop where SR Ca2+-reuptake can be expected to play a critical role [90]. Interestingly, in aged skeletal muscle where MAMs structure is partly impaired also shows alteration in SR Ca2+-reuptake. Li et al. highlight that SERCA1 activity declines markedly in aged skeletal muscle that induce mitochondrial Ca2+ overload leading to opening of the mPTP [61]. Studies using atrophy and sarcopenia models also highlighted altered SR Ca2+ reuptake especially near close proximity of MAMs [31]. Together, these findings position MAMs architecture as a finely tuned, bidirectional regulator of SR Ca2+ reuptake when balanced, but pathological when unbalance during conditions such as aging.

MAMs and ROS and ER/SR stress

Another signalling messenger that is closely associated with MAMs functionalities is ROS, which is primarily generated by mitochondria. ROS is a double-edged sword; on one hand some ROS is necessary for adaptive signaling, while excessive production drives pathology [96]. This balance is especially critical at MAMs, where even small shifts in mitochondrial ROS can disrupt communication inducing ER/SR stress. Under physiological conditions, low levels ROS enhance RyR1 opening amplifying Ca2+ release and supporting contractile performance [97]. MAM-microdomains contain antioxidant enzymes like superoxide dismutase and glutathione peroxidase, creating localized redox-regulated signaling hubs that manage oxidative stress and maintain Ca2+ channel activity. Chronic elevation of ROS disrupts this balance as found during aging. Excessive ROS oxidizes key tethering proteins, particularly MFN2 and IP3R, which destabilizes contact sites widening the gap between SR and mitochondria [82]. Simultaneously, ROS damage cardiolipin impairing ETC worsening mitochondrial dysfunction [72]. This creates a feed-forward cycle: chronic ROS oxidizes RyR1, causing SR Ca2+ leak and mitochondrial Ca2+ overload at remaining MAMs contacts, triggering further ROS production damaging more SR and MAMs proteins [96]. In conditions where this cycle persists for long (like sarcopenia), mitochondria floods with Ca2+ causing opening the mPTP, which triggers apoptosis [77]. This result in organellar dysfunction leading to reduced contractile force, prolonged muscle fatigue, and accelerated age-related decline in muscle function [31]. During aging it has been reported that levels of antioxidant enzymes, superoxide dismutase and glutathione peroxidise, at the MAMs decline weakening localized ROS scavenging [82]. Due to this oxidation of SERCA leading to faulty Ca2+-reuptake has also been reported to contribute to pathological progression during aging and/or atrophy [70, 97]. Under severe pathological states disrupted MAMs become the hub of ROS leading to accumulation of harmful proteins and lipids in the adjoining areas leading to lipotoxicity and SR stress.

MAMs remodelling during exercise and diseases

Emerging data has illustrated that MAMs in skeletal muscle are highly plastic structures whose molecular composition are continuously reshaped by physio-pathological stimuli [49]. Various types of exercise (acute vs chronic and endurance vs resistance) drive unique MAMs remodelling(Figure 4). While acute exercise functionally exploits pre-existing MAMs, chronic exercise training induces both structural and molecular remodelling. Endurance exercise improves mitochondria-triad association stability by enhancing expression of tether proteins such as MFN2, VDAC1, and GRP75 [90]. This supports efficient mitochondrial Ca2+ uptake and aligns ATP output with repeated contractile demand and increasing functionally competent interfaces that minimize Ca2+ leak and oxidative stress. In addition to MAMs remodelling, endurance training elevates expression of MCU and SERCA modulating Ca2+-microdomain homeostasis thereby bioenergetics [90]. Resistance exercise is suggested to promote mitochondrial biogenesis and fusion associated with increased MFN2 expression that would probably enhance MAMs stability [83]. These changes are often associated with RyR1 stabilization and reduced ROS leading to improved excitation-metabolism coupling. This coupling is best demonstrated by combined aerobic and resistance regimens that further optimize mitochondrial dynamics with increased OPA1, while reduced DRP1 expression [29]. Interestingly, literature report that benefits of caloric restriction and intermittent fasting rely on improved mitochondrial efficiency is partly due to remodelling of MAMs-related properties [50]. Further, diseases that exhibit gross alteration in intramyocellular Ca2+ homeostasis also show maladaptive MAM remodelling. Muscles from diabetic patients and animal models have shown complex and context-dependent MAMs remodeling [49]. In some diabetic models reduced SR-mitochondrial coupling and diminished IP3R-VDAC interactions have been observed, while in others aberrant MAMs structure and mitochondrial Ca2+-overload has been found. Skeletal muscles from various muscle dystrophies exhibit varying degrees of MAMs remodelling both in patients and animal models. In Duchenne muscular dystrophy muscles exhibit extreme MAMs remodelling. In dystrophic muscle, hyperactive IP3R-dependent Ca2+-signaling at MAMs and altered tethering promote sustained mitochondrial Ca2+-overload and fragmentation [61]. Collectively, these observations underscore that MAM remodeling in skeletal muscle exists along a broad continuum: exercise drives an adaptive recalibration of contact architecture that enhances metabolic flexibility, whereas disease states impose maladaptive remodeling that impair excitation-metabolic coupling accelerating functional decline.

5. Aging-Induced Disruption of Skeletal Muscle Homeostasis

Skeletal muscles undergo several small, but progressive remodelling during aging one of which is disruption of MAMs architecture and function. In sarcopenia, fast-twitch fibers are preferentially lost, with the slow-twitch fibers attempting to compensate through fast-type gene upregulation [17]. In fact, single-nucleus profiling of human fast-twitch fibers revealed transcriptional upregulation of inflammatory and atrophy-related pathways, while downregulation of protein synthesis and mitochondrial biogenesis genes [18]. Proteomic studies document a shift toward slow-type protein isoforms and accumulation of oxidized proteins, indicating chronic oxidative stress. Histological analyses confirmed preferential type II fiber atrophy, failed denervation-reinnervation cycles with modified NMJ, and expansion of intermuscular adipose and connective tissue. In sarcopenic muscle, stem cell populations decline with reduced protein synthesis and increased chemokine expression impairing regeneration [3]. Further, studies have shown association of sarcopenic hallmarks (like gait speed, grip strength, and chair-stand performance) with molecular signatures of Ca2+ dysregulation and mitochondrial dysfunction [18].

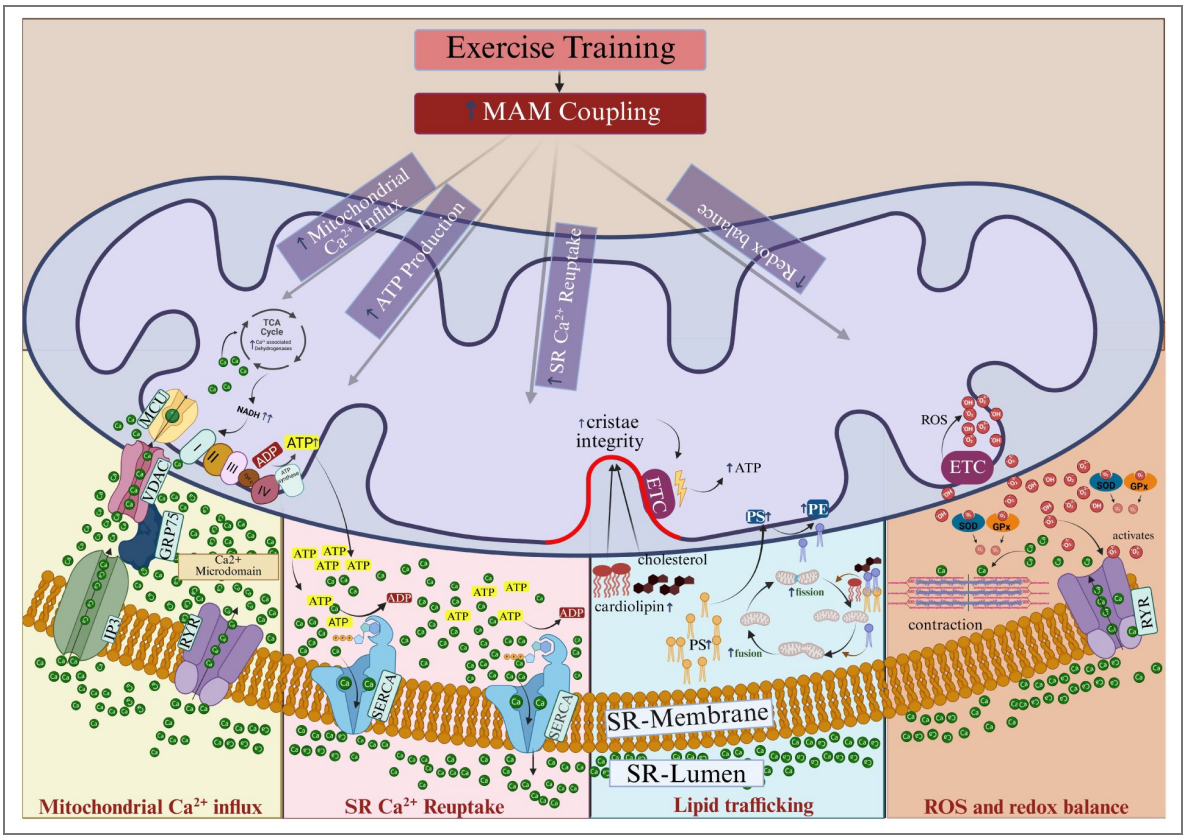

Figure 4. Beneficial effects of exercise are routed through MAMs functionality.

Exercise amplifies most of the functionalities of MAMs thereby enhancing the ATP production, Ca2+- crosstalk, lipid trafficking, and ROS handling capacity. Ca2+-microdomain intimacy including greater SR-mediated Ca2+-release and SERCA-based Ca2+-reuptake occur to a greater extent by exercise. Mitochondrial fusion occurs to a higher degree upon adaptation to exercise that require greater influx of lipids to maintain lipid composition. Exercise also is suggested to increase ROS production as well as its scavenging capacity especially near the MAMs. This figure was created using *BioRender.com*.

Impairments in Ca2+-Signaling

In sarcopenia, Ca2+ dysregulation has been shown as a central mechanism [19]. In young muscle, SR Ca2+ release and reuptake occur rapidly and efficiently, supporting normal contraction and relaxation. However, aging disrupts this balance through multiple converging mechanisms including expressional and posttranslational modifications of MAMs-related proteins. Downregulation of junctophilins widen SR-T-tubule gaps impairing voltage to Ca2+ signal conversion [82], while loss of FKBP12 (a RyR1 regulator) and DHPR subunit-switching (β1a increase while α1 decrease) reduce peak Ca2+ transient amplitude [97]. Simultaneously, expression of SERCA declines while micropeptide regulators SLN and PLB increase, slowing Ca2+ reuptake that disrupt the precise temporal coordination of Ca2+-microdomains at MAMs [23]. Post-translational oxidation of SERCA and RyR1 has also been reported in aged muscles that can contribute to dysregulation of Ca2+-microdomains and intracellular Ca2+-distribution [97]. The above changes in aged muscle along with T-tubular fragmentation and loss of triadic structure compromise Ca2+ release synchrony across fibers, weakening the privileged Ca2+- transfer through MAMs [24]. Altered Ca2+-microdomains homeostasis leads to mitochondrial Ca2+-overload amplifies mitochondrial dysfunction increasing ROS generation [31].

Mitochondrial Dysfunction

Another hallmark of aged skeletal muscle is impairment in mitochondrial networks and precise structural alignment with other organelles. Impaired mitophagy in aged muscle has been reported, which is suggested to stem from reduced PINK1/Parkin signaling and lysosomal dysfunction [65]. So, damaged mitochondria accumulate producing excess ROS that perturbs redox-sensitive proteins at SR-mitochondrial contact sites thereby destabilizing MAMs [96]. Simultaneously, mitochondrial biogenesis is suppressed due to downregulation of PGC-1α, NRF1, and NRF2 while inner-membrane organization is disrupted because of decreased cardiolipin supply [72]. These mechanisms collectively reduce oxidative phosphorylation capacity and ATP availability during contraction compromising muscle function and maintenance of SR-mitochondrial tethers [73]. Alterations in Ca2+ influx-efflux mechanisms have been linked to mitochondrial dysfunction; however, whether this dysfunction results from mitochondrial Ca2+ deficiency or pathological Ca2+ overload remains controversial [59]. It has been suggested ROS excess in aged muscle activate catabolic stress pathways including p38 MAPK and NF-κB while suppressing anabolic signaling (like mTOR) and NRF2-driven antioxidant response causing SR protein destabilization and MAMs dysregulation [96]. Further, circulating mitochondrial DNA and proteins released from apoptotic mitochondria increase local (intramuscular) inflammatory mediators that compromise SR/MAMs function and integrity [76]. These muscle structural functionality is highly flexible and has been shown to be reversed by interventions like pharmacotherapy (e.g. urolithin A, cardiolipin supplementation) and exercise [72]. Surprisingly however, the interventions become ineffective after the derailments have reached a threshold; therefore, early and sustained prevention strategies to maintain mitochondrial health in muscle have been proposed [73].

NMJ stability and Denervation

The plasticity of the skeletal muscle requires stability of NMJ and subsynaptic organellar structural arrangement. During muscle function, fibers undergo regular denervationreinnervation cycle, which reduces as aging progress. In aged muscles, surviving neurons attempt to reinnervate denervated fibers through compensatory sprouting, but failure of this result in permanent denervation that is suggested to be a cause of age-associated muscle loss [33]. It has been shown that expression and activity of proteins [such as agrin, MuSK(Muscle-Specific Kinase), and Lrp4(Low-density lipoprotein receptor-related protein 4)] that are key for maintaining NMJ stability decline with age [98]. In fact, circulating agrin fragments correlate with terminal Schwann cell loss and synaptic failure during muscle aging that may indicate association with impaired subsynaptic MAMs [99]. The stability of NMJ is also closely associated with neurotrophic factors (BDNF, GDNF, insulin-like growth factor-1), which support synaptic protein

synthesis essential for MAMs integrity [98]. The abundance of these factors reduces with age creating a permissive environment for degeneration of NMJ structure [33]. Also, denervation triggers secondary cascade of organellar dysfunction including SR fragmentation and disruption of mitochondrial architecture, critical for derailment of MAMs. Interestingly, exercise and electrical stimulation can partially preserve NMJ integrity by upregulating neurotrophic signalling that also help in retaining MAMs organization, especially during early stages [99]. Age-related increased denervation also causes SR fragmentation along with downregulation of Ca2+-handling proteins (RyR1, SERCA) which is often linked with disruption of subsynaptic SR-mitochondrial contacts [33]. These data indicate that age-induced NMJ remodelling in the skeletal muscle can be a critical influencer of MAMs functioning.

Lipid Accumulation

Intramuscular lipid accumulation, a hallmark of aging muscle, is driven by reduced mitochondrial capacity and altered lipid metabolism [100]. While lipids serve as fuel for oxidative muscles, altered mitochondrial activity leads to accumulation of intermediates like ceramides and diacylglycerols, which impairs insulin signaling, promotes inflammation destabilizing SR-mitochondrial contacts [100, 101]. Lipotoxic metabolites activate inflammatory pathways such as NF-kB and JNK, compounding oxidative stress and accelerating atrophy while simultaneously damaging MAM-tethering proteins [101]. These lipid droplets preferentially accumulate near mitochondria, physically disrupting MAMs formation impairing the efficiency of Ca2+ transfer between SR-mitochondria [32]. Proteomic studies reveal that lipid accumulation correlates directly with reduced MAMs density causing lipotoxicity and ER stress [49]. Intriguingly, aged muscles express PDK4 that suppress glucose oxidation reducing the metabolic flexibility needed for adapting to changing energy demands which relies on MAMs structural stability [26]. Exercise has been shown to improve muscle mitochondrial function and restore lipid handling capacity that reduces MAMs-related dysfunctions [64]. Interestingly, pharmacological lipid modulators; like PPAR agonists and AMPK activators or omega-3 supplementation, have been found to reduce intramyocellular lipotoxic stress markers leading to at least partial restoration of MAMs integrity [100].

Breakdown of SR-Mitochondrial Communication in Aging

The factors discussed above (Ca2+ dysregulation, mitochondrial dysfunction, NMJ instability and lipid accumulation) converge to create a state of profound structural fragility in aging muscle. Recent literature highlights loss of structural MAM integrity reduces Ca2+ coupling efficiency that jeopardize energy supply, further compromising contractile performance and muscle metabolic flexibility [50]. Disruption of MAMs structure is suggested to be a key factor in the pathogenesis of several muscle disorders like sarcopenia, atrophy, and age-related impaired injury (including post-exercise) recovery [31]. Under these pathological conditions tethering proteins of MAMs (like IP3R1, GRP75, VDAC1, and MFN2) exhibit either reduced abundance or mislocalization [82]. Interestingly, MAMs disruption is reported even before clear muscle atrophy or fiber-type changes manifest during aging [50]. Out of all the MAMs-tethering proteins role of MFN2 seems to be better demonstrated. Both aging and disuse-induced atrophy progressively decrease MFN2 levels, while MFN2 overexpression can partially rebuild MAMs structure and improve mitochondrial function in aged muscle [92]. However, overall strength is only modestly restored and full reversal in extreme cases remained elusive [83]. In aged-muscle fibers, disrupted MAMs lead to impairment in microdomain Ca2+-homeostasis leading to mitochondrial Ca2+-overload reducing ATP supply for sustained or repeated contractions [61]. In addition to Ca2+, MAMs disruption compromise essential lipid trafficking between SR and mitochondria (especially cardiolipin) that is key for ATP production capacity of mitochondria [72]. Surprisingly, impaired MAMs is suggested to undermine mitochondrial quality control due to malfunctioning of key fission-fusion regulators (OPA1, DRP1, MFN1/2) and mitophagy proteins (PINK1, Parkin) [65]. Some studies have suggested aging muscle also gains input from systemic metabolic dysfunction through crosstalk between muscle, adipose tissue, and bone (via myokines and adipokines), establishing chronic low-grade inflammation termed “inflammaging”, which further exacerbates MAMs deterioration [18].

6. Targeting MAMs to treat muscle health

Several strategies have been tested to improve metabolic health of the skeletal muscle providing beneficial outcome in metabolic disease treatment. Interestingly, some of these strategies target either preserving or restoring MAMs structure and function in the skeletal muscle [49, 50]. Exercise remains the most accessible and robust non-pharmacological approach. As discussed earlier exercise remodel MAMs through various mechanisms leading to reduction of ROS and preserving excitation-metabolism coupling [90]. Studies show that combined aerobic and resistance regimens optimize mitochondrial dynamics (increased OPA1, reduced DRP1) that support MAMs stability [29]. Multiple types of nutraceutical methods have been assessed to improve muscle health and fascinatingly, some of them exert their effects at least partly by manipulating MAMs functionalities [102]. Some nutraceuticals like resveratrol have been shown to modulate mitochondrial MAM Ca2+ microdomains and improve mitochondrial function in skeletal muscle [103, 104], while nicotinamide riboside elevates the muscle NAD+ metabolome and attenuates mitochondrial dysfunction in aged skeletal muscle [105, 106]. Branched-chain amino acids (BCAA) including leucine-enriched protein and betahydroxy beta-methylbutyrate (HMB) that sustain muscle protein synthesis also promote mitochondrial biogenesis and improve SR-mitochondrial Ca2+-crosstalk probably influencing MAMs integrity [107, 108]. Further, cardiolipin supplementation has been demonstrated to help in enhancing MAMs-related functions in both cardiac and skeletal muscles; depletion of cardiolipin has been specifically linked to sarcopenia and may represent a druggable pathway [109, 110]. Recently, various proteins having implication for MAMs Ca2+-microdomains have been exploited as pharmacological targets [50]. Among the tethering proteins, MFN2 has been targeted by mini-peptides and small-molecule agonists to restore MAMs architecture [111, 112]. SR Ca2+ mediators like SERCA and RyR1 have been targeted with CDN1163 (activator) and S107 (stabilizer) respectively to effectively modulate Ca2+-microdomains homeostasis to improve muscle function [113, 114]. Proteins of mitochondria side of MAMs have also been targeted. Notable ones are MCU sensitizers, which bolster mitochondrial Ca2+ uptake and ATP production at microdomains; but, at high doses exhibit the risk of mPTP opening and apoptosis [88]. Targeted mitochondrial antioxidants (such as MitoQ, SS-31) show ability to limit ROS-induced MAMs damage at appropriate dosing, while preserving adaptive ROS signaling [115, 116]. Further, agents that regulate mitochondrial biogenesis (like PGC-1a agonists) [117, 118] or mitophagy ("urolithin A" with/without cardiolipin supplementation) [119, 120] have been found to improve MAMs and mitochondrial flexibility thereby promoting muscle health. In addition, genetic manipulations of MAMs proteins have also been explored in the preclinical models. MFN2 overexpression and selective inhibition of excessive IP3R1-mediated mitochondrial Ca2+ flux have been evaluated [91, 92]. Further studies in human or human-derived primary cells will demonstrate if these approaches can be used as gene therapies offering organelle-specific corrections and MAMs architecture.

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Peer reviews

Reviewer #1 (Public review):

Summary:

This manuscript is a narrative review addressing age-related alterations in SR-mitochondria interactions in skeletal muscle and their contribution to sarcopenia. It synthesizes existing literature on calcium signaling, mitochondrial dynamics, redox balance, and structural remodeling, and discusses potential interventions including exercise and pharmacological strategies. While the topic is timely and relevant, the manuscript largely reiterates established concepts without providing sufficient conceptual novelty, critical synthesis, or mechanistic insight beyond the current literature.

Strengths:

(1) Timely topic: The focus on SR-mitochondria communication in aging muscle is relevant and of growing interest.

(2) Broad coverage: The review compiles a wide range of literature spanning calcium handling, mitochondrial biology, ROS signaling, and exercise physiology.

(3) Clear organization: The manuscript is structured logically with thematic sections (SR, mitochondria, MAMs, aging, interventions).

(4) Didactic value: Could serve as a general overview for non-specialists entering the field.

Weaknesses:

(1) Lack of novelty and conceptual advance: The manuscript does not offer new hypotheses, frameworks, or critical reinterpretation of the field. Most statements summarize already well-established knowledge, and no unifying model or novel perspective is developed to justify publication in a high-impact journal like eLife.

(2) Limited critical analysis: The review is predominantly descriptive rather than analytical. Conflicting findings (e.g., MFN2 roles, MAM density changes, Ca^{2+} overload vs deficiency) are mentioned but not critically evaluated or reconciled. There is little discussion of limitations in the cited studies or gaps in the field.

(3) Overgeneralization and speculative claims: Several assertions are presented with insufficient nuance (e.g., causal links between MAM disruption and sarcopenia, or therapeutic efficacy of interventions). The distinction between correlation and causation is often unclear, reducing scientific rigor.

(4) Insufficient depth for a specialist audience: Despite its length, the manuscript lacks mechanistic depth in key areas (e.g., precise molecular regulation of MAMs in vivo, tissue-specific differences, quantitative aspects of Ca^{2+} flux). It reads more like a textbook summary than a high-level scholarly review.

(5) Redundancy and verbosity: Many sections repeat similar concepts (Ca^{2+} dysregulation, ROS effects, mitochondrial dysfunction) without adding new insight, leading to an unnecessarily long manuscript with limited added value.

(6) Weak integration of recent literature into a coherent narrative: Although many references are cited, they are not effectively synthesized into a cohesive argument. The manuscript lacks a strong central thesis or clearly defined take-home messages.

(7) Limited translational or experimental perspective: The section on therapeutic targeting is largely speculative and does not critically assess feasibility, limitations, or current clinical evidence.

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Reviewer #2 (Public review):

This review addresses a highly relevant and timely topic, namely the role of sarcoplasmic reticulum-mitochondria communication and mitochondria-associated membranes (MAMs) in skeletal muscle aging. The manuscript successfully brings together literature from several interconnected fields, including calcium signaling, mitochondrial biology, excitation-contraction coupling, muscle metabolism, and sarcopenia. Given the growing interest in organelle crosstalk as a determinant of muscle health and disease, the topic is undoubtedly of considerable interest to the readership and has the potential to make a valuable contribution to the field.

However, in its current form, the manuscript devotes a substantial proportion of its content to the description of well-established concepts that are already extensively covered in the literature. Large sections are dedicated to general skeletal muscle physiology, excitation-contraction coupling, calcium handling, mitochondrial biology, and MAM structure and composition. While this background information is useful, the level of detail is often excessive for a review that aims to focus on aging-induced alterations in SR-mitochondria interactions. As a consequence, the central theme of the manuscript becomes diluted, and the review reads more like a broad overview of skeletal muscle physiology than a focused analysis of aging-related MAM remodeling.

In contrast, the sections specifically dedicated to aging and MAM dysfunction, which represent the most novel and potentially impactful aspects of the review, are comparatively brief and largely descriptive. The discussion of how aging alters MAM architecture, calcium microdomains, mitochondrial calcium signaling, and organelle communication would benefit from substantially greater depth. For example, although the manuscript highlights alterations in proteins such as MFN2, IP3R, VDAC, and MCU, the mechanistic implications of these changes for sarcopenia and age-associated muscle dysfunction are not critically developed. Similarly, the review would be strengthened by a more comprehensive discussion of the evidence linking MAM disruption to impaired muscle performance, metabolic inflexibility, denervation, and mitochondrial dysfunction during aging.

Another limitation is that much of the manuscript summarizes published findings without sufficiently evaluating the strength of the evidence or discussing existing controversies. Several statements imply causal relationships between MAM disruption and sarcopenia, whereas in many cases, the available data remain largely correlative. The authors should more clearly distinguish between established mechanisms, experimental observations, and emerging hypotheses. A more critical assessment of conflicting findings, particularly regarding the role of MFN2 and the dual consequences of altered mitochondrial calcium uptake, would considerably improve the scientific rigor of the review.

A major omission concerns the role of mitochondrial Ca^{2+} uptake in skeletal muscle physiology and aging. Throughout the manuscript, mitochondrial Ca^{2+} uptake is presented as a central determinant of muscle function and as a key mechanism linking MAM disruption to sarcopenia. However, the authors do not adequately discuss evidence that challenges this view. In particular, genetic mouse models lacking MCU exhibit surprisingly mild skeletal muscle phenotypes under basal conditions despite a near-complete abolition of rapid mitochondrial Ca^{2+} uptake. These findings have generated considerable debate regarding the physiological importance of mitochondrial Ca^{2+} uptake for muscle function and metabolic regulation. While MCU deletion clearly affects exercise adaptation and certain stress responses, the relatively modest baseline phenotype suggests the existence of compensatory pathways and raises important questions regarding the extent to which impaired mitochondrial Ca^{2+} uptake alone can explain age-associated muscle dysfunction. A balanced review should acknowledge these observations and discuss the ongoing debate regarding the

relative contributions of mitochondrial Ca^{2+} deficiency versus mitochondrial Ca^{2+} overload in aging skeletal muscle.

Similarly, the discussion of MFN2 would benefit from greater nuance. The manuscript largely presents MFN2 as a structural tether linking the sarcoplasmic reticulum and mitochondria. However, MFN2 is a multifunctional protein with well-established roles in mitochondrial fusion, mitochondrial network organization, mitophagy regulation, and metabolic signaling. Consequently, many of the phenotypes associated with altered MFN2 expression cannot be unequivocally attributed to changes in MAM formation. The review does not sufficiently distinguish between the effects of MFN2 on organelle tethering and its effects on mitochondrial dynamics. This distinction is particularly important because several studies have questioned whether MFN2 acts primarily as a positive tether, a negative regulator of contacts, or whether its influence on organelle communication is secondary to its role in controlling mitochondrial morphology. As a result, attributing age-related alterations in SR-mitochondria communication solely to changes in MFN2-mediated tethering may oversimplify a considerably more complex biological scenario.

The manuscript's organization could also be improved. The sections discussing aging-related alterations, mitochondrial dysfunction, calcium dysregulation, oxidative stress, and therapeutic interventions contain significant overlap and repetition. Streamlining some background sections and reallocating space to a more detailed discussion of aging-specific mechanisms would help maintain focus and improve readability. In particular, the manuscript would benefit from expanding the sections on aging-induced MAM remodeling, age-dependent changes in MAM composition and ultrastructure, and the potential of MAM-targeted interventions as therapeutic strategies for sarcopenia.

Finally, the review would gain from a stronger future perspectives section. Several important questions remain unresolved, including whether MAM disruption is a primary driver of muscle aging or a secondary consequence of mitochondrial dysfunction, how MAM architecture differs among muscle fiber types during aging, and whether MAM-associated proteins could serve as reliable biomarkers or therapeutic targets in human sarcopenia. Highlighting these knowledge gaps would further enhance the review's impact.

Overall, the manuscript covers an important and emerging area of research and contains a valuable compilation of the relevant literature. Nevertheless, substantial revision is required to reduce the emphasis on well-established background information, deepen and critically analyze the aging-specific sections, and provide a more focused discussion of the role of MAMs in skeletal muscle aging and sarcopenia.

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