



The contribution of mitochondria to age-related skeletal muscle wasting: A sex-specific perspective

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ABSTRACT

As people age, their skeletal muscle (SkM) experiences a decline in mitochondrial functionality and density, which leads to decreased energy production and increased generation of reactive oxygen species. This cascade of events, in turn, might determine the loss of SkM mass, strength and quality. Even though the mitochondrial processes dysregulated by aging, such as oxidative phosphorylation, mitophagy, antioxidant defenses and mtDNA transcription, are the same in both sexes, mitochondria age differently in the SkM of men and women. Indeed, the onset and magnitude of the impairment of these processes seem to be influenced by sex-specific factors. Sexual hormones play a pivotal role in the regulation of SkM mass through both genomic and non-genomic mechanisms. However, the precise mechanisms by which these hormones regulate mitochondrial plasticity in SkM are not fully understood. Although the presence of estrogen receptors in mitochondria is recognized, it remains unclear whether androgen receptors affect mitochondrial function. This comprehensive review critically dissects the current knowledge on the interplay of sex in the aging of SkM, focusing on the role of sex hormones and the corresponding signaling pathways in shaping mitochondrial plasticity. Improved knowledge on the sex dimorphism of mitochondrial aging may lead to sex-tailored interventions that target mitochondrial health, which could be effective in slowing or preventing age-related muscle loss.

1. Introduction

Aging could be accompanied by a progressive loss of skeletal muscle (SkM) mass, strength and quality, a condition known as sarcopenia [1]. This muscle disorder has a prevalence of 10–50 % among the aging population and affects both men and women [2,3]. However, the onset, duration, and magnitude of age-related muscle wasting may be sex-specific due, at least in part, to the role of sex hormones in the regulation of SkM homeostasis [4]. The anabolic effect of testosterone on SkM health has been extensively studied. Estrogens also play a protective role in SkM mass and function by decreasing inflammation. However, the mechanisms underlying estrogen effects on SkM are less well known

than those of testosterone (reviewed by [3,5]).

SkM fiber atrophy varies by fiber type, being type II more prone to age-related wasting [6]. This type of fiber is more susceptible to denervation [7], which seems to occur in aged muscles [8], following which reinnervation can take place. Nevertheless, reinnervated fibers within older SkMs adopt a distinct phenotype [7], which is characterized by the co-expression of multiple myosin heavy chain (MHC) isoforms and type I fiber grouping [9]. Type I fibers are highly oxidative, and so are more reliant on mitochondrial health status to support contraction [10]. However, age-related accumulation of dysfunctional mitochondria is an acknowledged contributor to SkM wasting, a process that typically initiates around the age range of 40 to 50 years [11,12]. Impaired ATP

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generation and overproduction of reactive oxygen species (ROS) are associated with the loss of mitochondrial functionality, and trigger the activation of intracellular signaling pathways, such as those mediated by the Forkhead box O (FoxO) transcription factor family proteolytic pathway. These pathways might inhibit the Akt anabolic pathway, and stimulate the release of mitochondrial DNA (mtDNA) with consequent activation of the NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome, and apoptosis. These events culminate in the loss of SkM mass and function [13]. Given that mitochondrial function and biogenesis are regulated by a variety of factors, including sex hormones [5], age-related SkM mitochondria remodeling is likely affected by sex hormones, contributing to the sexual dimorphism observed in age-related sarcopenia. Nonetheless, sex-specific SkM mitochondrial adaptations during aging are poorly understood. In the pursuit of understanding the impact of sexual dimorphism on mitochondrial remodeling in the context of age-related SkM adaptation, VOSviewer tool was employed to visualize and analyze bibliometric data related to the concurrent terms “age,” “sex,” “skeletal muscle,” and “mitochondria”. The outcomes, as illustrated in Fig. 1, underscore that research on SkM wasting predominantly centers around mitochondrial remodeling. It is worth noting that a limited number of these studies delve into the influence of sex, or even employ female animal models of

aging for their investigations.

This review aims to critically examine the current knowledge on the impact of sex on SkM aging focusing on the role played by sex hormones and the corresponding mediated signaling pathways in shaping mitochondrial plasticity.

2. Sex differences in skeletal muscle aging: the molecular perspective

The recognition of sex as a pivotal biological variable to take into consideration in wasting conditions is gaining momentum. Nevertheless, experimental insights into sarcopenia are sometimes contradictory, which may be explained, at least in part, by the experimental models used for the study of this condition. Being a chronic disease of the elderly, sarcopenia takes years to develop in humans. Due to the extended time course and ethical concerns surrounding the acquisition of SkM biopsies from potentially frail patients, researchers have turned to experimental models, mainly rodents, to unravel the molecular mechanisms underlying SkM aging. Still, there are shortcomings in their application to the study of sarcopenia. For instance, rodents and humans are different in the fiber type composition of muscles with type IIb fibers being present in rodents SkM but absent in humans [14]. Unlike humans

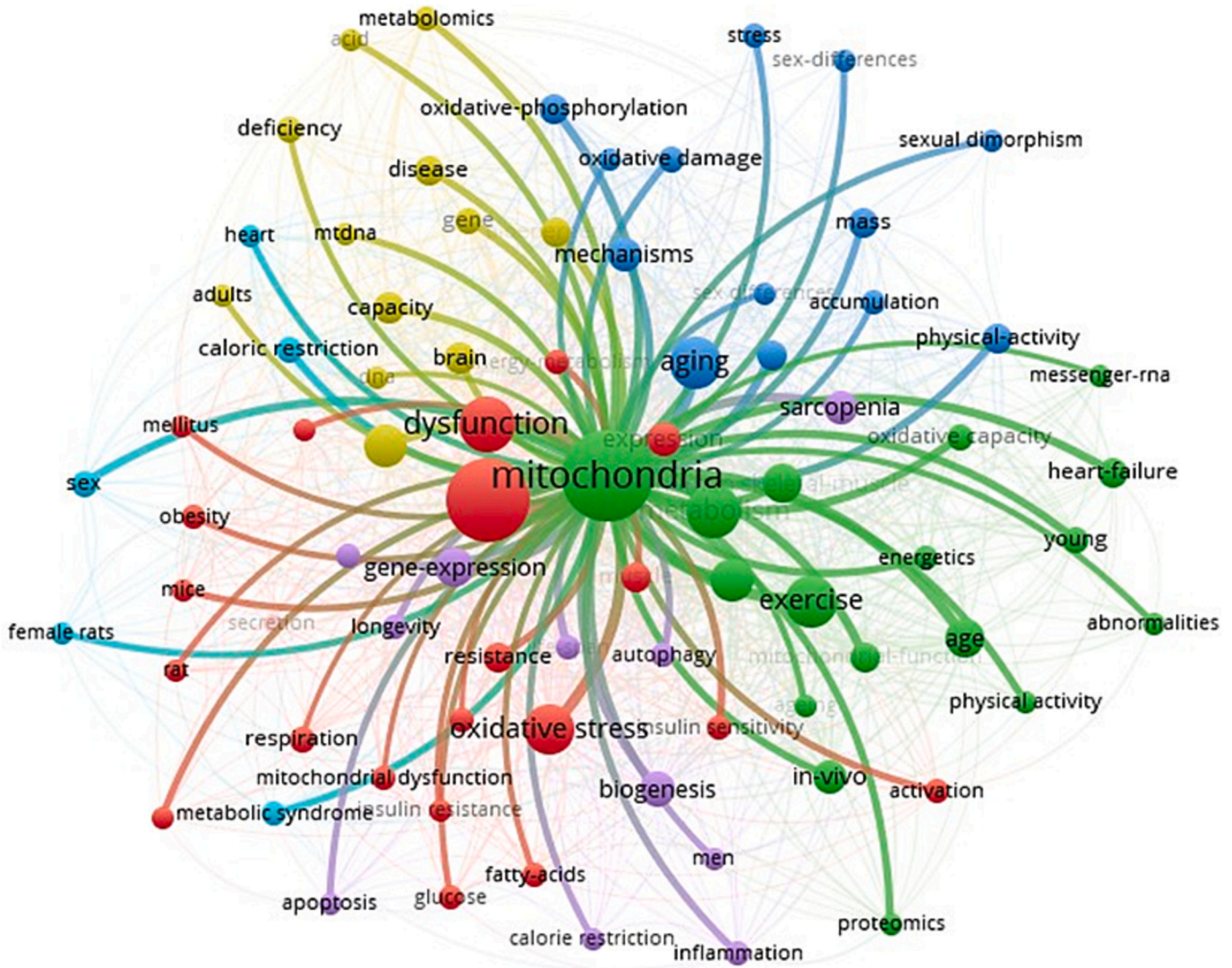


Fig. 1. Network analysis reveals common terms related to aging, sex, skeletal muscle adaptation, and mitochondrial remodeling. Node size corresponds to the term's frequency, while edges indicate co-occurring terms. Different colors represent clusters of associated terms. This co-occurrence network visualization map was generated using VOSviewer.

and rats, mice do not express aromatase in extragonadal tissues, which is a chief source of estrogen production in men and postmenopausal women [15]. Additionally, differences between human and rodent reproductive senescence are apparent. Unlike the occurrence of ovarian failure in women, adult neoogenesis has been observed in rodents (reviewed by [14]). These disparities should be considered when juxtaposing the physiological and molecular adaptations occurring in sarcopenia in humans and animal models.

In their pioneer study, Coggan et al. [16] compared SkM aging across both sexes in humans. They found no significant differences in the proportion of different fiber types in the *gastrocnemius* or the activity of some enzymes, such as glycogen phosphorylase or lactate dehydrogenase (LDH), between both young and old men, as well as between young and old women. Still, the cross-sectional area (CSA) of type I fibers becomes larger while the CSA of Iia and Iid/x fibers become significantly smaller with aging, in both men and women. Another study unveiled no differences in phosphofructokinase (PFK) activity in SkM due to aging; however, it was higher in women's SkM than in men's, suggesting a more glycolytic phenotype in women. Furthermore, significantly thinner *gastrocnemius* muscles were observed in aged women and men, as evaluated through ultrasound measurement, compared with their counterparts in the 20s; however, the age-related decline in *gastrocnemius* muscle thickness initiated ten years earlier in men (in their 50s)

than in women (in their 60s) [17].

In searching for the molecular basis underlying sex differences in age-related muscle wasting, sex hormones appear as the most probable candidates in the regulation of key molecular pathways of the SkM with an impact on its mass and functionality [18]. Testosterone is known to promote muscle protein synthesis and muscle regeneration through its androgen receptor (AR) [19]. The mechanisms of estrogen action are less well characterized in SkM but seem to involve the regulation of metabolism through the estrogen receptors (ER) α or ER β . For instance, estrogen regulates the expression of the glucose transporter (GLUT)4 through ERs, in particular ER α [20]. Androgens also regulate the expression of this transporter. Indeed, when testosterone and dehydroepiandrosterone (DHEA) were added to cultured myotubes, GLUT4 expression and translocation to the cell membrane increased [21] (Fig. 2). Alterations in glucose homeostasis with aging seem to be more pronounced in females than in males, despite the greater loss of SkM in males [20].

Overall, the effects of sex hormones on SkM are mediated by the activity of their receptors through genomic and non-genomic pathways, which have been found to be altered in age-related muscle wasting [22].

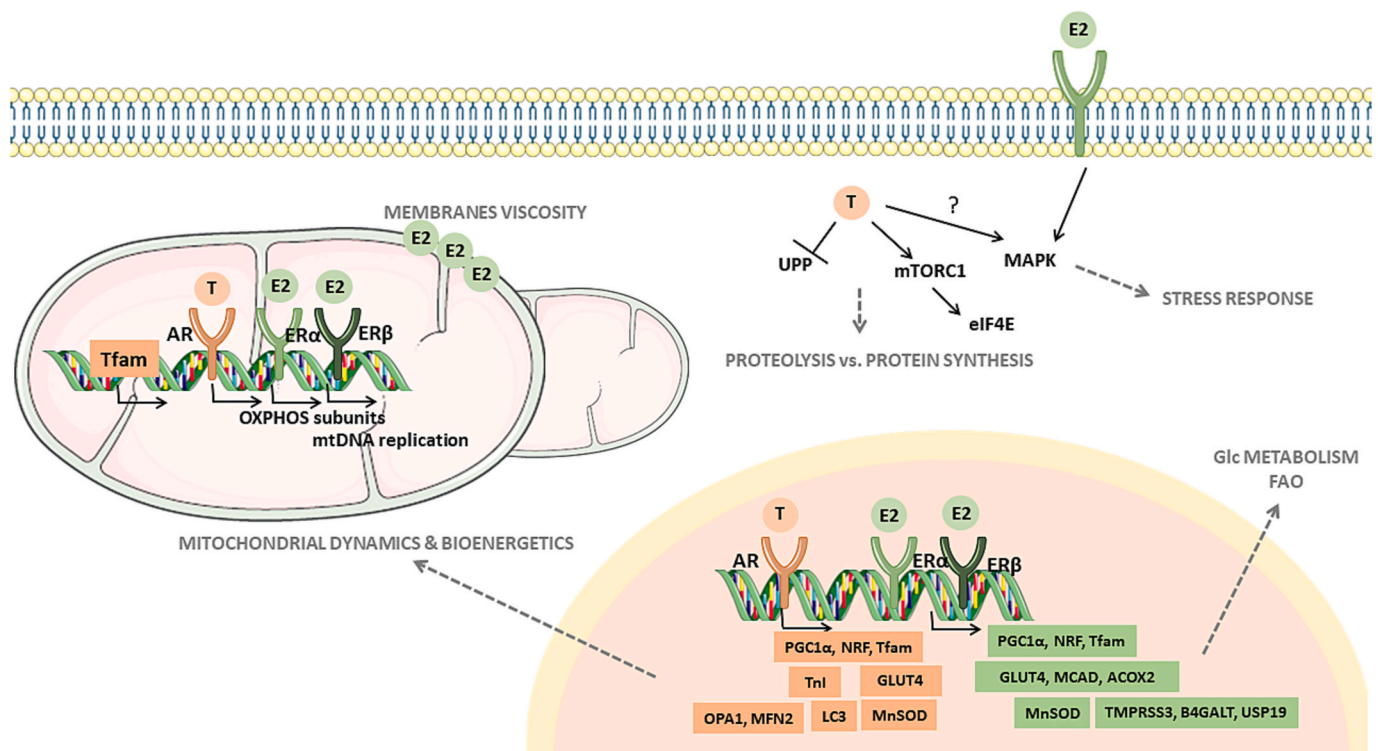


Fig. 2. Effects of sex hormones in skeletal muscle remodeling highlighting the signaling pathways modulated by 17 β -estradiol (E2) and testosterone (T) on mitochondrial sculpting and protein turnover. E2 and T boost the expression of PGC1 α and NRF, consequently elevating Tfam expression. Within the mitochondria, this transcription factor governs the expression of OXPHOS subunits encoded by mtDNA. This promotes mitochondrial biogenesis and enhances antioxidant defenses. T also exerts control over mitochondrial dynamics by regulating the expression of genes encoding proteins involved in fusion, fission, and auto(mito)phagy. Moreover, E2 and T can directly modulate mtDNA replication and transcription by binding to their receptors located within the mitochondria. E2 and T regulate SkM metabolism by stimulating Glc uptake through GLUT4. Additionally, E2 enhances FAO, correlating with increased mitochondrial biogenesis. Furthermore, E2 and, eventually, T, can activate MAPK through a non-genomic pathway, reinforcing mitochondrial antioxidant defenses. T enhances SkM mass through mTOR pathway activation while inhibiting the UPP. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

Abbreviations: ACOX2, peroxisomal acyl-coenzyme A oxidase 2; AR, androgen receptor; B4GALT, beta-1,4-galactosyltransferase 1; E2, eIF4E, eukaryotic translation initiation factor 4E; ER, estrogen receptor; FAO, fatty acid oxidation; Glc, glucose; GLUT4, glucose transporter 4; LC3, protein 1 light chain 3; MAPK, mitogen-activated protein kinase; MCAD, medium-chain acyl-CoA dehydrogenase; MFN2, mitofusin 2; MnSOD, manganese superoxide dismutase; mTORC1, mechanistic target of rapamycin complex 1; mtDNA, mitochondrial DNA; NRF, nuclear respiratory factor; OPA1, optic atrophy 1; OXPHOS, oxidative phosphorylation; PGC-1 α , peroxisome proliferator-activated receptor-gamma coactivator-1 alpha; Tfam, mitochondrial transcriptional factor A; TMPRSS3, transmembrane protease serine 3; TnI, troponin I; UPP, ubiquitin-proteasome pathway; USP19, ubiquitin specific protease 19.

2.1. Androgen-centered pathways

Testosterone circulates like a prohormone and can originate active hormones like dihydrotestosterone (DHT) and 17 β -estradiol (E2). Androgens can be converted into E2 and estrone (E1) by the enzyme aromatase, which is expressed in humans and rats (but not in mice) SkM [5,23,24]. After binding to their receptor, androgens regulate the expression of proteins involved in glucose and protein metabolism (genomic pathway). Testosterone treatment of C2C12 cells (mouse myoblast line) was found to increase the mRNA expression of genes encoded by mtDNA like *NADH dehydrogenase subunits 1 and 4* and to induce the expression of the nuclear respiratory factors 1 and 2 (Nrf-1 and Nrf-2) and the mitochondrial transcription factors A (Tfam) and B2 (TFB2M), which suggests that this hormone plays a role in the regulation of mitochondrial transcription and biogenesis in the SkM [25] (Fig. 2).

Regarding protein synthesis, distinct roles of androgens have been reported. For instance, the administration of testosterone to male subjects (aged between 19 and 40 years) was reported to augment protein synthesis in the SkM [26]; however, this increase was not supported by an enhancement of amino acid transport into the SkM. Thus, protein synthesis can be conceivably upregulated through an efficient reutilization of amino acids coming from protein breakdown [27]. Also, an improvement in muscle anabolism and strength was induced by testosterone administration in old men (≥ 60 years old) [28]. In vitro studies with C2C12 cells exposed to DHT and testosterone showed no response, probably due to their low concentration of AR [29]. When the same cell line was engineered to increase AR expression, an increase in total protein amount was observed in response to DHT and testosterone treatment, confirming the anabolic action of androgens on muscle fibers [29].

Testosterone also impacts protein synthesis in the SkM by interplaying with the mammalian target of rapamycin complex 1 (mTORC1), a key factor in the regulation of protein synthesis and, thus, of muscle mass [30]. In mice *gastrocnemius* and cultured C2C12 myotubes, testosterone loss was found to reduce protein synthesis through downregulation of Akt/mTORC1 signaling. Castrated mice exhibited reduced *gastrocnemius* muscle mass, body weight and strength [31]. mTORC1 is downregulated by Regulated in development and DNA damage response 1 (REDD1) and AMP-activated protein kinase (AMPK), which is a key sensor of the cell metabolic state [32]. When phosphorylated, mTORC1 modulates mRNA translation by acting on eukaryotic translation initiation factor (eIF)4E [33]. Androgens could also modulate mitogen-activated protein kinase (MAPK) signaling, another pathway involved in a wide variety of cellular processes, such as proliferation, differentiation, and stress response [34]. Steroid hormones can activate the MAPK pathway before exerting their action in the nucleus [35]. In fact, in rat myotubes, testosterone was found to enhance ERK1/2 phosphorylation on Thr²⁰² and Tyr²⁰⁴ [36]. Conversely, underphysiological concentrations of testosterone (induced by castration) were associated with the increased content of atrogenes (MuRF1 and MAFbx), which are muscle-specific E3 ligases from the ubiquitin-proteasome pathway (UPP). Atrogenes are important regulators of the SkM mass and fiber size and higher expression of atrogenes has been reported during catabolic stimuli leading to muscle wasting [37].

Androgen-induced alterations in protein turnover were associated with changes in the SkM phenotype. For instance, the inhibition of the AR pathway in AR knockout mice led to the decrease of the protein levels of the troponin I specific for slow-twitch fibers and an increase of the troponin T specific for fast-twitch fibers in the *quadriceps*, showing the role of AR in SkM fiber types remodeling towards a more oxidative and mitochondria-dependent metabolism [38]. Treatment of isolated intact mammalian SkM fiber bundles with physiological concentrations of DHT was found to increase both twitch and tetanic contractions in fast-twitch fibers and decrease them in slow-twitch fibers. The authors proposed that DHT treatment activated the epidermal growth factor receptor (EGFR), leading to the switch-on of ERK1/2 function in both

fiber types and, consequently, to phosphorylation of myosin light chain (MLC) kinase, which in turn phosphorylated the 20 kDa regulatory MLCs, culminating in the fiber type-specific outcomes. However, testosterone treatment did not affect force and increased ERK1/2 phosphorylation in slow-twitch fibers [39].

Overall, androgens have a significant impact on SkM mass and force generation, which seems to be linked to enhanced glucose metabolism and protein synthesis while downregulating the UPP signaling. Both genomic and non-genomic pathways are involved in modulating these effects of androgens on SkM remodeling.

2.2. Estrogen-centered pathways

Estrogens control different cellular processes, such as differentiation and proliferation, similarly to androgens. These cholesterol-derived molecules, when unbound, are capable of diffusing across cell membranes directly into the cytoplasm and bind intracellular or membrane-linked receptors. There are three known estrogen receptors (ER), ER α , ER β and G protein-coupled receptor (GPER), all present in the SkM [5]. After dimerizing, the estrogen-ER complex goes into the nucleus and regulates the transcription of estrogen-responsive genes, which encode for proteases, metabolic enzymes (e.g. peroxisomal acyl-coenzyme A oxidase 2 (ACO2), transmembrane protease serine 3 (TMPRSS3), beta-1,4-galactosyltransferase 1 (B4GALT1)), among others. These data highlight the pivotal role of estrogens in regulating the expression of genes that encode proteins crucial for an array of cellular functions, spanning from metabolism to tumor suppression [40].

Estrogens can also act through a non-genomic pathway by binding to a membrane ER and activating signaling proteins [41] (Fig. 2). Like androgens, estrogens play a role in the regulation of protein synthesis in the SkM; however, the exact mechanisms by which these hormones operate are not clear. Changes in estrogen levels during the menstrual cycle do not seem to affect the rate of protein synthesis and muscle contractibility since during the luteal phase, when estrogen concentration is higher than in the follicular phase, no changes in myofibrillar protein synthesis were observed [42]. During menopause, the decrease in E2 and progesterone (P4) concentrations was associated with an increase in the basal rate of muscle protein synthesis [43]. Some evidence suggests that hormone replacement therapy (HRT) may have small but significant benefits in preventing the loss of SkM mass and strength following menopause, but the data are scarce and sometimes contradictory, probably influenced by various factors such as HRT dosage, timing of therapy administration post-menopause, dietary factors, physical activity levels, age, and treatment durations (reviewed by [44]).

Regarding protein breakdown, some studies support the role of estrogens in the repression of atrogenes, thus preserving SkM mass [44,45]. In fact, a net negative protein balance was reported in the *vastus lateralis* of post-menopausal women, probably caused by an upregulation of catabolic genes like *FOXO3A* and *MuRF1* [46]. Curiously, E2 upregulated the deubiquitinating enzyme ubiquitin-specific peptidase 19 (USP19) mRNA expression in the *soleus* and *gastrocnemius* muscles of female ovariectomized mice and decreased the ratio of muscle mass-to-body weight [47]. Also, an E2 dose-dependent increase in USP19 mRNA expression was observed in the myoblast cell line C2C12 [47]. Nuclear ER α was suggested to be involved in the upregulation of USP19 expression in the presence of E2 [48]. Force impairment was found in the *soleus* muscle in female skmER α KO mice characterized by specific deletion of the *ER α* gene in the SkM. However, fiber type distribution did not differ between *soleus* and *gastrocnemius* muscles of skmER α KO and skmER α WT female mice, suggesting the involvement of other ER types [49]. Several studies confirm the positive role of E2 in the regulation of muscles' strength [50–52]. Furthermore, it has been suggested that the decrease in estrogen levels may be associated with an increase of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF α) and interleukin (IL)-6, which might contribute to sarcopenia

development [53]. Still, more studies are needed to clearly establish the role of E2 in the onset of age-related muscle wasting.

3. Role of mitochondrial remodeling in skeletal muscle aging

Experimental evidence highlights the key role of mitochondrial health in the preservation of SkM function during aging, to which the maintenance of mitochondrial integrity and dynamics (i.e., the balance between fusion and fission) contributes [13]. Mitochondrial protein quality control (mtPQC) is the first line for the upkeep of mitochondrial proteostasis [54]. Several proteases are involved in mtPQC, including the ATP-dependent mitochondrial protease LONP1. In LONP1 knockout mice, severe mitochondrial dysfunction was reported and associated with reduced muscle fiber size and strength, a hallmark of an early aging phenotype [54], indicating a possible relation between LONP1 and sarcopenia. Other mtPQC proteins also impact SkM remodeling. For instance, mice lacking fusion proteins, such as mitofusin (Mfn)-1 and -2, display mitochondrial dysfunction, accumulation of mtDNA damage, and a severe deficit in growth. Deletion of both Mfn-1 and -2 in adult mice SkM resulted in a decrease in exercise performance, indicating that mitochondrial fusion is essential for muscle functionality [55]. Accelerated sarcopenia was reported in mice lacking Mfn-2 [56]; however, it is still unclear if fusion proteins decrease during aging. Indeed, conflicting results have been reported, with some studies showing the upregulation of both fusion and fission-related proteins during aging and others reporting no changes in their expression levels [57,58].

In addition to fusion and fission, mitophagy, a fundamental mechanism conserved from yeast to humans, regulates mitochondrial quality and quantity [59], playing a key role in muscle aging with studies reporting a downregulation of mitophagy-related proteins in the SkM of old rodents [60,61]. In fact, the amount of the E3 ubiquitin ligase Parkin, a key player in mitophagy, was reported to be lower in the *tibialis anterior* and *gastrocnemius* muscles of old mice (18 months; sex not specified) [62] and in the *gastrocnemius* of male rats (24 months) [63]. On the other hand, overexpression of Parkin was found to induce SkM mass and size gain, being associated with improved activity of the oxidative phosphorylation (OXPHOS) complexes II and IV [62].

These alterations in mitochondrial dynamics in aged SkM seem to be related to the loss of mtDNA-encoded proteins. Accumulation of mtDNA deletions was found to be comparable in old subjects with and without sarcopenia [64]. Nevertheless, the 12,175–13,708 bp deletion in the mitochondrially encoded ND5 region, which is a hotspot for mtDNA deletions and associated with aging and disease states, was observed only in old subjects with sarcopenia. However, this deletion encompassing the coding region of the OXPHOS complex I subunits had no impact on the activity of this complex since no differences were observed between sarcopenic and non-sarcopenic old subjects. Curiously, significant differences were observed in OXPHOS complex IV activity, which was higher in the SkM of old sarcopenic subjects [64].

SkM fibers are rich in mitochondria, the primary producers of ROS particularly during resting periods. The deleterious consequences of increased ROS generation have been closely associated with SkM aging. Augmented ROS and/or reactive nitrogen species (RNS) production acts as a trigger for the oxidative damage of mitochondrial biomolecules [65]. The susceptibility of mitochondria to ROS-induced damage was found associated with enhanced mitochondrial protein carbonylation (marker of protein oxidation) in biopsies of the *vastus lateralis* of old non-sarcopenic and old sarcopenic subjects, as compared with younger ones. Moreover, this increased protein carbonylation was correlated with a reduction in muscle strength [66]. Elevated levels of protein carbonylation have been consistently observed during the aging process across different muscles (e.g. external intercostal muscles, *vastus lateralis*, and *gastrocnemius*), in both animal models [67,68] and human studies [69,70]. The increase in mitochondrial protein carbonylation, primarily affecting crucial components of the citric acid cycle and OXPHOS, was

reported to be closely associated with the reduced capacity for ATP production in the hind limb SkMs of aged mice [71]. The loss of activity of these proteins due to oxidation is expected to negatively impact SkM metabolism and, consequently, contraction, possibly contributing to the loss of muscle mass and strength. In addition, the age-related overproduction of ROS has been associated with mtDNA damage, impaired mitochondrial biogenesis, and apoptosis [66].

ROS/RNS could be neutralized by several antioxidant systems to avoid their reaction with macromolecules, and consequent damage to the cell [72,73]. One of the main antioxidant systems in SkM is the mitochondrial superoxide dismutase (MnSOD) [72]. Higher levels of MnSOD were observed in the *quadriceps* and external intercostal muscles of old subjects (68 ± 5 years old) compared to young ones (25 ± 4 years old) [69]. These results might suggest a concerted effort to neutralize the heightened ROS levels. Indeed, the deficiency of this protein in type IIb fibers was associated with increased oxidative stress and reduced muscle mass, albeit in young adult mice [74]. When comparing the activity of MnSOD between women and men, both young (<35 years old) and old (≥70 years old), no sex-related differences were found in the *vastus lateralis* [75]. However, higher levels of MnSOD were observed in the external intercostal muscles of elderly women in comparison with young women, a difference not found in the men groups [69]. Interestingly, higher levels of lipid peroxidation and oxidized glutathione (GSSG) were seen in SkM from men compared to women [76]. Another important set of antioxidant enzymes in SkM includes catalase and glutathione peroxidase (GPx), which catalyzes the breakdown of hydrogen peroxide (H₂O₂) in peroxisomes and mitochondria, respectively. Research on catalase activity has shown some variations based on muscle type and age. For instance, catalase activity was found unchanged in the *vastus lateralis*, *rectus abdominis*, and *gluteus maximus* of old individuals (65–90 years old) compared to young counterparts (<65 years old) [73]. Nevertheless, the intercostal and *quadriceps* muscles of old humans (68 ± 5 years old) exhibited an increased catalase content compared with young ones (25 ± 4 years old) [69]. However, diminished levels of catalase, as well as reduced levels of GPx, were observed in the *quadriceps* of aged animals that presented increased H₂O₂ levels [77]. Recently, in a mouse model of accelerated sarcopenia, it was demonstrated that SkM overexpressing catalase targeting mitochondria effectively reduces ROS production in the *gastrocnemius* muscle, restoring ROS levels to those seen in control animals. This overexpression was associated with preserved SkM mass and force generation, as well as a partial inhibition of the decrease in fiber size [78], underscoring the potential pivotal role of mitochondrial catalase in preventing age-related muscle wasting. Peroxiredoxins (Prdx) also respond to H₂O₂, surpassing other antioxidant enzymes in reactivity to ROS. Within the Prdx family, Prdx3 stands out by eliminating approximately 90 % of H₂O₂ within mitochondria, playing a pivotal role in preserving mitochondrial homeostasis. This, in turn, has significant implications for SkM contractile functions [79,80]. Indeed, the depletion of this antioxidant enzyme resulted in accelerated apoptosis [81]. Conversely, the overexpression of Prdx3 has been shown to rescue sarcopenic SkM by mitigating mitochondrial H₂O₂ generation and enhancing mitochondrial function, counteracting the decline in both muscle quantity and quality [82].

Although all mitochondria serve a similar function in providing ATP to the SkM, their morphological and biochemical characteristics vary according to their location within the myofiber. Intermyofibrillar mitochondria (IMF), the ones embedded among the myofibrils, have higher OXPHOS complexes content and activity, and respiratory rates. Subsarcolemmal mitochondria (SS) present a higher content of chaperones [83,84]. Both these populations are impacted by aging. In the EDL of male rats, both IMF and SS volumes decrease with age [85]. However, in the *gastrocnemius* of 88–96-week-old mice, larger, less circular SS mitochondria and longer, more branched IMF mitochondria are described compared to young ones (8–12-week-old). These changes seem to result from a change in the fusion/fission balance towards

increased fusion, but its role in the regional adaptation of mitochondria to aging remains unexplored [86]. Moreover, citrate synthase activity has been reported to be 30–50 % higher in IMF than SS mitochondria, with no age-related differences. COX activity is also higher in IMF mitochondria, and aging has no noticeable effect [87]. Additionally, the increase in biogenesis in response to chronic electric stimulation is lower in old rats (36 months-old) compared to young rats (6 months-old). The adaptations of old mitochondria to chronic stimulation do not significantly differ based on fiber location [85].

Overall, the age-related decline in mitochondrial functionality is characterized by impaired OXPHOS activity, leading to excessive ROS/RNS generation and, thus, to the damage of biomolecules and cellular structures, irrespective of mitochondria location within the muscle fiber. Aging muscles seem to have a reduced capacity to eliminate these damaged biomolecules, which become toxic thereby perpetuating a vicious cycle that ultimately determines the fate of muscle fibers.

4. Skeletal muscle mitochondria age differently in men and women

SkM aging is thought to be associated with reduced mitochondrial density and functionality, with an impact on fiber metabolic choices [13,88]; however, the aging-induced alterations on mitochondria appear to be distinct between sexes. While a decline in mitochondria content and an enlargement of intramyocellular lipid droplets have been documented in older adults (69–73 years) in comparison to their younger counterparts (21–25 years), the distribution of lipid droplets in contact with mitochondria diverges between sexes. This is lowest in the subsarcolemmal region among older men and extends throughout the entirety of the fiber area among older women [89], which might potentially mirror distinct metabolic adaptations of SkM to the aging process. Mitochondria from the *gastrocnemius* of female rats display a higher mtDNA and protein content *per* muscle mass compared to male ones [90]. Increased amount of antioxidant systems (MnSOD and GPx) in females' mitochondria was linked with a lower susceptibility to oxidative damage [91]. Moreover, older adults, particularly women, have a decreased IMF mitochondrial size (reported in *vastus lateralis*), which was inversely and directly related to isometric tension and myosin-actin cross-bridge kinetic, respectively [88].

The molecular process harbored at mitochondria and more susceptible to aging is the OXPHOS pathway [92,93]. Increased levels of complexes II and V were observed during aging in the *quadriceps* muscle but only in females. Moreover, the analysis of the respiratory function showed a decline in functionality in aged mice, with this decline being more pronounced in old females than in old males. In both sexes, there was an increase in the levels of certain markers of auto(mito)phagy, such as BNIP3, Parkin, Beclin-1, and ATG7 [94]. Nevertheless, conflicting data have been reported in other studies [60,61,63]. The findings from Triolo et al. [94] may indicate the presence of dysfunctional lysosomes, which could potentially limit the capacity for mitophagic breakdown. Noticeably, female mice exhibited a higher abundance of auto(mito)phagy proteins, as well as a greater index of basal SkM autophagosome clearance compared to male mice, potentially attributed to a higher mitochondrial density. This impaired auto(mito)phagy was associated with an age-related decline in the complex-I and complex-II active respiration, with a more pronounced effect observed in females than males. Therefore, compromised auto(mito)phagy may lead to the accumulation of dysfunctional organelles, contributing to the excessive production of ROS that characterizes sarcopenia [95]. Since ROS are key players in SkM aging and mitochondria their main producers, the activity of antioxidant systems from 120 muscle biopsies (*vastus lateralis*, *rectus abdominis* and *gluteus maximus*), 57 from men and 63 from women, was evaluated [76]. The authors reported an age-related increase in MnSOD activity in women and a decrease in total SOD activity in men, which was accompanied by an augmented protein carbonyl content in older men. These authors concluded that SkM from men appear to be

more susceptible to age-related oxidative stress than those from women. Also, a decrease in catalase and glutathione S-transferase (GST) activities was reported in *vastus lateralis* biopsies during aging [75]. While for GST sex differences were reported, with higher activity in old women than in men, no significant differences emerged for catalase activity. SOD and GPx activities were also evaluated, and no age- or sex-related differences were observed. However, the analyses were conducted in whole muscle and were not directly associated with mitochondrial number or volume.

Generally, mitochondria age differently in men's and women's SkM; however, the mitochondrial processes dysregulated by aging in both sexes are mainly OXPHOS, mitophagy, antioxidant defenses and mtDNA transcription. The onset and severity of these processes' impairment distinguish the metabolic and functional alterations in SkM among sexes during aging (overviewed in Fig. 3). Despite these observed differences, robust data on the specific effects of sex on SkM mitochondrial aging are still lacking.

5. Effects of sex hormones in mitochondrial remodeling

The precise role of sex hormones in regulating mitochondrial function is not well understood, particularly in the SkM. Genetic elements regulated by sex hormones were already discovered in mtDNA, for both ER and AR [96]. Moreover, ER α , ER β , and AR have been detected in mitochondria using subcellular fractionation followed by immunoblotting and microscopy techniques in C2C12 cells and SkM tissue [97–99]. Mass spectrometry (MS)-based proteome analysis of mitochondria also confirmed the presence of ER β in this organelle while data regarding localization of AR in mitochondria remain less clear [100]. Intriguingly, neither ERs or AR are included among the 1136 human and 1140 mouse genes encoding proteins with the strong support for mitochondrial localization (MitoCarta3.0; www.broadinstitute.org/mitocarta/mitocarta30-inventory-mammalian-mitochondrial-proteins-and-pathways, assessed in September 2023). This may be due to either the lack of validation of MS data or the potential contamination of the mitochondrial fractions obtained following subcellular fractionation with other cellular components.

The presence of ERs and AR in mitochondria suggests the involvement of sex hormones in the regulation of different mitochondrial functions. Since they regulate the expression of mtDNA genes, sex hormones may influence the bioenergetics and remodeling of mitochondria. For instance, young women had higher SkM mitochondrial volume density and increased capacity for fatty acid and lactate oxidation than young men [101]. A higher mitochondrial calcium uptake capacity has been observed in female compared to male fast-twitch muscle. This difference may be attributed to the greater IMF mitochondrial volume density, at least in the *tibialis anterior* [102]. Additionally, the *gastrocnemius* of female rats displayed higher mtDNA and Tfam levels, and OXPHOS complexes activities, *per* muscle mass, than male animals [90]. Several genes linked to mitochondrial function exhibited varying levels of expression in the human *vastus lateralis* concerning sex, including PGC-1 α and citrate synthase. This finding is in line with the well-documented sex differences in muscle fiber composition, where females typically possess a higher proportion of type I fibers, known for their more oxidative characteristics. In fact, over 5000 isoforms of gene products display differential expression between males and females, explaining differences in phenotype and susceptibility to age-related SkM mass loss [103]. From these, E2 upregulates the transcript levels of several mtDNA genes encoding for subunits from OXPHOS complexes with an impact on mitochondrial respiratory function [104]. This effect may entail the upregulation of PGC-1 α and NRF1, which, in turn, promote the transcription of Tfam. This transcription factor binds and enhances the expression of mtDNA-encoded genes [105] (Fig. 2). These results suggest that SkM mitochondria exhibit a sexual dimorphism that might explain why females adapt more easily to altered metabolic energy situations.

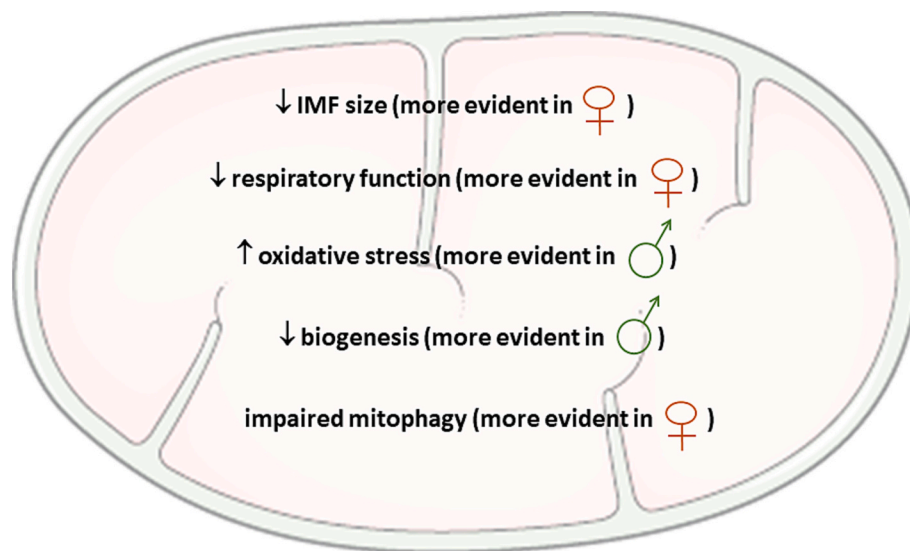


Fig. 3. Overview of sex differences in mitochondrial remodeling during skeletal muscle aging. The impact of aging is highlighted, emphasizing the sex where these effects are more pronounced. The skeletal muscle phenotype in females exhibits greater oxidative characteristics than in males, owing to a higher mitochondrial density. IMF mitochondria appear to be more vulnerable to the effects of aging when compared to SS mitochondria, with a more pronounced reduction in size observed in females. Female exhibited an age-related lower respiratory function whereas male mitochondria demonstrate lower mtDNA content per muscle mass and lower PGC-1 α and Tfam levels, potentially accounting for decreased mitochondrial biogenesis. Conflicting data regarding the contribution of mitophagy to mitochondrial remodeling in SkM has been reported, with some studies suggesting an increase of this process, potentially related to dysfunctional lysosomes, which appear to be more prominent in females. The augmented content of antioxidant defenses renders female mitochondria less susceptible to the age-related increase of oxidative stress compared to their male counterparts. These mitochondrial alterations seem to mirror the direct and indirect effects of age-related fluctuations in the levels of sex hormones in both sexes. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

Abbreviations: IMF, intermyofibrillar.

In muscle-specific ER α knockout (MERKO) mice, both impaired glucose homeostasis and diminished muscle oxidative metabolism and ROS scavenging capacity were observed. Mitochondria of these animals were less densely distributed in the subsarcolemmal compartment compared with control animals [106]. Moreover, a reduction in the *soleus* expression of mtDNA polymerase γ 1 (Poly 1) and mtDNA-directed RNA polymerase (*Polrmt*) was found in MERKO animals, indicating that ER α regulates mtDNA replication [106]. Nevertheless, ER β has been reported as the prevailing isoform within mitochondria (reviewed by [96]). This receptor has been demonstrated to interact with Hsp27, a chaperone located within mitochondria, and to mediate the protective effects of E2 in C2C12 cells [107]. Another study demonstrated the presence of E2 within the mitochondrial membrane, where it directly modulates the biophysical properties and bioenergetic function of SkM mitochondria. E2 was found to decrease microviscosity of mitochondria membranes, independently of its receptor. This had significant consequences, as short-term ovariectomy decreased mitochondrial respiratory function, cellular redox state, and insulin sensitivity [108].

Unlike estrogens, the knowledge about androgens and their effects on mitochondria is scarce. The overexpression of specific mtDNA genes, in particular those encoding COX2a and COX3a, the two mitochondrial subunits of COXIV, was observed in SkM after administration of testosterone at low physical activity levels. For the other OXPHOS complexes, an increase in activity was proven but it was not accompanied by the overexpression of their subunits as in the case of complex I [109]. These authors also showed that testosterone treatment influences mitochondrial fusion and fission by upregulating the expression of genes involved in the regulation of mitochondrial dynamics, such as *MFN2* and *OPA1*, and by increasing the content of mitophagy proteins, such as LC3AII and LC3BII (Fig. 2). Likewise, in another study, testosterone administration increased PGC1 α , ATP5B and COXIV protein content in the *gastrocnemius* muscle, and mRNA levels of genes involved in mitochondrial biogenesis, namely *PGC1 α* , *NRF1*, *NRF2* and *Tfam* in C2C12 myotubes [110]. Furthermore, young women exposed to testosterone

presented increased capillary-to-fiber ratio, specific mitochondrial respiratory flux activating complex I, linked complex I and II, uncoupled respiration and electron transport system capacity of the *vastus lateralis* muscle compared to the placebo group [111]. These data suggest that testosterone promotes mitochondrial biogenesis and functionality.

Overall, sex hormones may impact mitochondrial morphology and functionality by acting directly or indirectly on this organelle; however, most of the evidence on the effects of sex hormones in mitochondria is circumstantial, particularly in SkM, and more experiments are needed. Moreover, it is not yet known whether the aforementioned alterations are due to differences in SkM composition in terms of oxidative vs. glycolytic fibers between male and female.

6. Conclusions

SkM does not age equally in men and women. Given the significant role of mitochondria in SkM aging, there is a need to pinpoint the role of sex in driving age-related mitochondrial remodeling. Experimental evidence supports sex specificity in this organelle's aging, due to direct and indirect effects of sex hormones. In fact, despite not being listed in MitoCarta, several studies place ERs and AR in mitochondria to mediate some of the effects of sex hormones on muscle fibers. Age-related decreasing levels of these hormones, mostly E2, promote mtDNA encoded OXPHOS complexes subunits' downregulation and activity, and increased susceptibility of mitochondrial biomolecules to oxidative damage. Mitochondrial subpopulations seem to be differently modulated by sex, and these subpopulations' specific adaptations trigger the age-related decline of muscle performance. Still, sex differences in SkM aging are poorly understood. Further research should integrate bioenergetic, structural and molecular data of SkM mitochondria from both sexes (including pre- and post-menopausal women) not only to get more insight into age-related sarcopenia but also to extend such knowledge to various wasting conditions, including cachexia. This multifaceted approach will prompt the development of therapeutic interventions

tailored to mitochondrial sex specificity.

CRedit authorship contribution statement

Alessandro Nuccio: Writing – original draft. **Rita Nogueira-Ferreira:** Writing – review & editing, Visualization. **Alexandra Moreira-Pais:** Writing – review & editing, Visualization. **Alessandro Attanzio:** Writing – review & editing, Supervision. **José Alberto Duarte:** Writing – review & editing, Conceptualization. **Claudio Luparello:** Writing – review & editing, Supervision. **Rita Ferreira:** Writing – review & editing, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- [1] A.J. Cruz-Jentoft, G. Bahat, J. Bauer, et al., Sarcopenia: revised European consensus on definition and diagnosis, *Age Ageing* 48 (1) (2019) 16–31, <https://doi.org/10.1093/ageing/afy169>.
- [2] S.K. Papadopoulou, Sarcopenia: a contemporary health problem among older adult populations, *Nutrients* 12 (5) (2020) 1293, <https://doi.org/10.3390/nu12051293>.
- [3] B.M. Roberts, K.M. Lavin, G.M. Many, et al., Human neuromuscular aging: sex differences revealed at the myocellular level, *Exp. Gerontol.* 106 (2018) 116–124, <https://doi.org/10.1016/j.exger.2018.02.023>.
- [4] L.J. Anderson, H. Liu, J.M. Garcia, Sex differences in muscle wasting, in: F. Mauvais-Jarvis (Ed.), *Sex and Gender Factors Affecting Metabolic Homeostasis, Diabetes and Obesity*, Springer International Publishing, Cham, 2017, pp. 153–197, https://doi.org/10.1007/978-3-319-70178-3_9.
- [5] S.L. McMillin, E.C. Minchew, D.A. Lowe, E.E. Spangenburg, Skeletal muscle wasting: the estrogen side of sexual dimorphism, *Am. J. Physiol. Cell Phys.* 322 (1) (2022) C24–c37, <https://doi.org/10.1152/ajpcell.00333.2021>.
- [6] S. Ciciliot, A.C. Rossi, K.A. Dyar, B. Blaauw, S. Schiaffino, Muscle type and fiber type specificity in muscle wasting, *Int. J. Biochem. Cell Biol.* 45 (10) (2013) 2191–2199, <https://doi.org/10.1016/j.biocel.2013.05.016>.
- [7] Y.N. Kwon, S.S. Yoon, Sarcopenia: neurological point of view, *J. Bone Metab.* 24 (2) (2017) 83–89, <https://doi.org/10.11005/jbm.2017.24.2.83>.
- [8] S.L. Rowan, K. Rygiel, F.M. Purves-Smith, N.M. Solbak, D.M. Turnbull, R. T. Hepple, Denervation causes fiber atrophy and myosin heavy chain co-expression in senescent skeletal muscle, *PLoS One* 7 (1) (2012), e29082, <https://doi.org/10.1371/journal.pone.0029082>.
- [9] R. Rudolf, M.M. Khan, S. Labelit, M.R. Deschenes, Degeneration of neuromuscular junction in age and dystrophy, *Front. Aging Neurosci.* 6 (2014) 99, <https://doi.org/10.3389/fnagi.2014.00099>.
- [10] P. Dowling, S. Gargan, D. Swandulla, K. Ohlendieck, Fiber-type shifting in sarcopenia of old age: proteomic profiling of the contractile apparatus of skeletal muscles, *Int. J. Mol. Sci.* 24 (3) (2023), <https://doi.org/10.3390/ijms24032415>.
- [11] D. Arsalan, B. Parvin, G. Meysam, R. Kamal, Mitochondrial biogenesis in skeletal muscle: exercise and aging, in: C. Julianna (Ed.), *Skeletal Muscle*, IntechOpen, Rijeka Ch. 10, 2012, <https://doi.org/10.5772/48411>.
- [12] V. Pesce, A. Cormio, F. Fracasso, et al., Age-related mitochondrial genotypic and phenotypic alterations in human skeletal muscle, *Free Radic. Biol. Med.* 30 (11) (2001) 1223–1233, [https://doi.org/10.1016/s0891-5849\(01\)00517-2](https://doi.org/10.1016/s0891-5849(01)00517-2).
- [13] J.P. Leduc-Gaudet, S.N.A. Hussain, E. Barreiro, G. Gouspillou, Mitochondrial dynamics and mitophagy in skeletal muscle health and aging, *Int. J. Mol. Sci.* 22 (15) (2021), <https://doi.org/10.3390/ijms22158179>.
- [14] W.Q. Xie, M. He, D.J. Yu, et al., Mouse models of sarcopenia: classification and evaluation, *J. Cachexia. Sarcopenia Muscle* 12 (3) (2021) 538–554, <https://doi.org/10.1002/jcsm.12709>.
- [15] H. Zhao, E.K. Pearson, D.C. Brooks, et al., A humanized pattern of aromatase expression is associated with mammary hyperplasia in mice, *Endocrinology* 153 (6) (2012) 2701–2713, <https://doi.org/10.1210/en.2011-1761>.
- [16] A.R. Coggan, R.J. Spina, D.S. King, et al., Histochemical and enzymatic comparison of the gastrocnemius muscle of young and elderly men and women, *J. Gerontol.* 47 (3) (1992) B71–B76, <https://doi.org/10.1093/geronj/47.3.B71>.
- [17] K. Fujiwara, H. Asai, H. Toyama, et al., Changes in muscle thickness of gastrocnemius and soleus associated with age and sex, *Aging Clin. Exp. Res.* 22 (1) (2010) 24–30, <https://doi.org/10.1007/bf03324811>.
- [18] V. Dubois, M. Laurent, S. Boonen, D. Vanderschueren, F. Claessens, Androgens and skeletal muscle: cellular and molecular action mechanisms underlying the anabolic actions, *Cell. Mol. Life Sci.* 69 (10) (2012) 1651–1667, <https://doi.org/10.1007/s00018-011-0883-3>.
- [19] R.J. Urban, Y.H. Bodenbun, C. Gilkison, et al., Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis, *Am. J. Physiol. Endocrinol. Metab.* 269 (5 Pt 1) (1995) E820–E826, <https://doi.org/10.1152/ajpendo.1995.269.5.E820>.
- [20] B.J. Gheller, E.S. Riddle, M.R. Lem, A.E. Thalacker-Mercer, Understanding age-related changes in skeletal muscle metabolism: differences between females and males, *Annu. Rev. Nutr.* 36 (2016) 129–156, <https://doi.org/10.1146/annurev-nutr-071715-050901>.
- [21] K. Sato, M. Iemitsu, K. Aizawa, R. Ajisaka, Testosterone and DHEA activate the glucose metabolism-related signaling pathway in skeletal muscle, *Am. J. Physiol. Endocrinol. Metab.* 294 (5) (2008) E961–E968, <https://doi.org/10.1152/ajpendo.00678.2007>.
- [22] M. Velders, P. Diel, How sex hormones promote skeletal muscle regeneration, *Sports Med.* 43 (11) (2013) 1089–1100, <https://doi.org/10.1007/s40279-013-0081-6>.
- [23] K. Aizawa, M. Iemitsu, S. Maeda, N. Mesaki, T. Ushida, T. Akimoto, Endurance exercise training enhances local sex steroidogenesis in skeletal muscle, *Med. Sci. Sports Exerc.* 43 (11) (2011) 2072–2080, <https://doi.org/10.1249/MSS.0b013e31821e9d74>.
- [24] A.A. Larionov, D.A. Vasylyev, J.I. Mason, A.F. Howie, L.M. Berstein, W.R. Miller, Aromatase in skeletal muscle, *J. Steroid Biochem. Mol. Biol.* 84 (4) (2003) 485–492, [https://doi.org/10.1016/s0960-0760\(03\)00059-1](https://doi.org/10.1016/s0960-0760(03)00059-1).
- [25] L. Pronsato, L. Milanese, A. Vasconsuelo, Testosterone induces up-regulation of mitochondrial gene expression in murine C2C12 skeletal muscle cells accompanied by an increase of nuclear respiratory factor-1 and its downstream effectors, *Mol. Cell. Endocrinol.* 500 (2020), 110631, <https://doi.org/10.1016/j.mce.2019.110631>.
- [26] R.C. Griggs, W. Kingston, R.F. Jozefowicz, B.E. Herr, G. Forbes, D. Halliday, Effect of testosterone on muscle mass and muscle protein synthesis, *J. Appl. Physiol.* (1985) 66 (1) (1989) 498–503, <https://doi.org/10.1152/jappl.1989.66.1.498>.
- [27] A.A. Ferrando, K.D. Tipton, D. Doyle, S.M. Phillips, J. Cortiella, R.R. Wolfe, Testosterone injection stimulates net protein synthesis but not tissue amino acid transport, *Am. J. Physiol. Endocrinol. Metab.* 275 (5) (1998) E864–E871, <https://doi.org/10.1152/ajpendo.1998.275.5.E864>.
- [28] A.A. Ferrando, M. Sheffield-Moore, C.W. Yeckel, et al., Testosterone administration to older men improves muscle function: molecular and physiological mechanisms, *Am. J. Physiol. Endocrinol. Metab.* 282 (3) (2002) E601–E607, <https://doi.org/10.1152/ajpendo.00362.2001>.
- [29] Y. Chen, N.K. Lee, J.D. Zajac, H.E. MacLean, Generation and analysis of an androgen-responsive myoblast cell line indicates that androgens regulate myotube protein accretion, *J. Endocrinol. Invest.* 31 (10) (2008) 910–918, <https://doi.org/10.1007/bf03346441>.
- [30] O.A. Adegoke, A. Abdullahi, P. Tavajohi-Fini, mTORC1 and the regulation of skeletal muscle anabolism and mass, *Appl. Physiol. Nutr. Metab.* 37 (3) (2012) 395–406, <https://doi.org/10.1139/h2012-009>.
- [31] J.P. White, S. Gao, M.J. Puppa, S. Sato, S.L. Welle, J.A. Carson, Testosterone regulation of Akt/mTORC1/FoxO3a signaling in skeletal muscle, *Mol. Cell. Endocrinol.* 365 (2) (2013) 174–186, <https://doi.org/10.1016/j.mce.2012.10.019>.
- [32] J.L. Steiner, D.H. Fukuda, M.L. Rossetti, J.R. Hoffman, B.S. Gordon, Castration alters protein balance after high-frequency muscle contraction, *J. Appl. Physiol.* (1985) 122 (2) (2017) 264–272, <https://doi.org/10.1152/jappphysiol.00740.2016>.
- [33] I. Ben-Sahra, B.D. Manning, mTORC1 signaling and the metabolic control of cell growth, *Curr. Opin. Cell Biol.* 45 (2017) 72–82, <https://doi.org/10.1016/j.cob.2017.02.012>.
- [34] Y.J. Guo, W.W. Pan, S.B. Liu, Z.F. Shen, Y. Xu, L.L. Hu, ERK/MAPK signalling pathway and tumorigenesis, *Exp. Ther. Med.* 19 (3) (2020) 1997–2007, <https://doi.org/10.3892/etm.2020.8454>.
- [35] T. Simoncini, A.R. Genazzani, Non-genomic actions of sex steroid hormones, *Eur. J. Endocrinol.* 148 (3) (2003) 281–292, <https://doi.org/10.1530/eje.0.1480281>.
- [36] M. Estrada, A. Espinosa, M. Müller, E. Jaimovich, Testosterone stimulates intracellular calcium release and mitogen-activated protein kinases via a G protein-coupled receptor in skeletal muscle cells, *Endocrinology* 144 (8) (2003) 3586–3597, <https://doi.org/10.1210/en.2002-0164>.

- [37] D. Taillandier, C. Polge, Skeletal muscle atrogens: from rodent models to human pathologies, *Biochimie* 166 (2019) 251–269, <https://doi.org/10.1016/j.biochi.2019.07.014>.
- [38] S. Altuwajiri, D.K. Lee, K.H. Chuang, et al., Androgen receptor regulates expression of skeletal muscle-specific proteins and muscle cell types, *Endocrine* 25 (1) (2004) 27–32, <https://doi.org/10.1385/endo.25:1:27>.
- [39] M.M. Hamdi, G. Mutungi, Dihydrotestosterone activates the MAPK pathway and modulates maximum isometric force through the EGF receptor in isolated intact mouse skeletal muscle fibres, *J. Physiol.* 588 (Pt 3) (2010) 511–525, <https://doi.org/10.1113/jphysiol.2009.182162>.
- [40] K. Nishi, W. Fu, R. Kiyama, Novel estrogen-responsive genes (ERGs) for the evaluation of estrogenic activity, *PLoS One* 17 (8) (2022), e0273164, <https://doi.org/10.1371/journal.pone.0273164>.
- [41] R. Kiyama, Y. Wada-Kiyama, Estrogenic endocrine disruptors: molecular mechanisms of action, *Environ. Int.* 83 (2015) 11–40, <https://doi.org/10.1016/j.envint.2015.05.012>.
- [42] B.F. Miller, M. Hansen, J.L. Olesen, et al., No effect of menstrual cycle on myofibrillar and connective tissue protein synthesis in contracting skeletal muscle, *Am. J. Physiol. Endocrinol. Metab.* 290 (1) (2006) E163–e168, <https://doi.org/10.1152/ajpendo.00300.2005>.
- [43] G.I. Smith, J. Yoshino, D.N. Reeds, et al., Testosterone and progesterone, but not estradiol, stimulate muscle protein synthesis in postmenopausal women, *J. Clin. Endocrinol. Metab.* 99 (1) (2014) 256–265, <https://doi.org/10.1210/jc.2013-2835>.
- [44] A. Pellegrino, P.M. Tiidus, R. Vandenboom, Mechanisms of estrogen influence on skeletal muscle: mass, regeneration, and mitochondrial function, *Sports Med.* 52 (12) (2022) 2853–2869, <https://doi.org/10.1007/s40279-022-01733-9>.
- [45] M.L. Maltais, J. Desroches, I.J. Dionne, Changes in muscle mass and strength after menopause, *J. Musculoskelet. Neuronal Interact.* 9 (4) (2009) 186–197.
- [46] U. Raue, D. Sliwka, B. Jemiolo, C. Hollon, S. Trappe, Proteolytic gene expression differs at rest and after resistance exercise between young and old women, *J. Gerontol. A Biol. Sci. Med. Sci.* 62 (12) (2007) 1407–1412, <https://doi.org/10.1093/gerona/62.12.1407>.
- [47] M. Ogawa, R. Yamaji, Y. Higashimura, et al., 17 β -estradiol represses myogenic differentiation by increasing ubiquitin-specific peptidase 19 through estrogen receptor α , *J. Biol. Chem.* 286 (48) (2011) 41455–41465, <https://doi.org/10.1074/jbc.M111.276824>.
- [48] M. Ogawa, T. Kitakaze, N. Harada, R. Yamaji, Female-specific regulation of skeletal muscle mass by USP19 in young mice, *J. Endocrinol.* 225 (3) (2015) 135–145, <https://doi.org/10.1530/joe-15-0128>.
- [49] B.C. Collins, T.L. Mader, C.A. Cabelka, M.R. Inigo, E.E. Spangenburg, D.A. Lowe, Deletion of estrogen receptor α in skeletal muscle results in impaired contractility in female mice, *J. Appl. Physiol.* 124 (4) (2018) 980–992, <https://doi.org/10.1152/jappphysiol.00864.2017>.
- [50] D.L. Enns, P.M. Tiidus, The influence of estrogen on skeletal muscle: sex matters, *Sports Med.* 40 (1) (2010) 41–58, <https://doi.org/10.2165/11319760-000000000-00000>.
- [51] J. Hatae, Effects of 17 β -estradiol on tension responses and fatigue in the skeletal twitch muscle fibers of frog, *Jpn. J. Physiol.* 51 (6) (2001) 753–759, <https://doi.org/10.2170/jjphysiol.51.753>.
- [52] A.L. Moran, G.L. Warren, D.A. Lowe, Removal of ovarian hormones from mature mice detrimentally affects muscle contractile function and myosin structural distribution, *J. Appl. Physiol.* 100 (2) (2006) 548–559, <https://doi.org/10.1152/jappphysiol.01029.2005>.
- [53] V. Messier, R. Rabasa-Lhoret, S. Barbat-Artigas, B. Elisha, A.D. Karelis, M. Aubertin-Leheudre, Menopause and sarcopenia: a potential role for sex hormones, *Maturitas* 68 (4) (2011) 331–336, <https://doi.org/10.1016/j.maturitas.2011.01.014>.
- [54] Z. Xu, T. Fu, Q. Guo, et al., Disuse-associated loss of the protease LONP1 in muscle impairs mitochondrial function and causes reduced skeletal muscle mass and strength, *Nat. Commun.* 13 (1) (2022) 894, <https://doi.org/10.1038/s41467-022-28557-5>.
- [55] H. Chen, M. Vermulst, Y.E. Wang, et al., Mitochondrial fusion is required for mtDNA stability in skeletal muscle and tolerance of mtDNA mutations, *Cell* 141 (2) (2010) 280–289, <https://doi.org/10.1016/j.cell.2010.02.026>.
- [56] D. Sebastián, E. Soriano, J. Segalés, et al., Mfn2 deficiency links age-related sarcopenia and impaired autophagy to activation of an adaptive mitophagy pathway, *EMBO J.* 35 (15) (2016) 1677–1693, <https://doi.org/10.15252/emboj.201593084>.
- [57] G. Distefano, R.A. Standley, J.J. Dubé, et al., Chronological age does not influence ex-vivo mitochondrial respiration and quality control in skeletal muscle, *J. Gerontol. A Biol. Sci. Med. Sci.* 72 (4) (2017) 535–542, <https://doi.org/10.1093/gerona/glw102>.
- [58] E. Koltai, N. Hart, A.W. Taylor, et al., Age-associated declines in mitochondrial biogenesis and protein quality control factors are minimized by exercise training, *Am. J. Phys. Regul. Integr. Comp. Phys.* 303 (2) (2012) R127–R134, <https://doi.org/10.1152/ajpregu.00337.2011>.
- [59] M. Onishi, K. Yamano, M. Sato, N. Matsuda, K. Okamoto, Molecular mechanisms and physiological functions of mitophagy, *EMBO J.* 40 (3) (2021), e104705, <https://doi.org/10.15252/emboj.2020104705>.
- [60] L. García-Prat, M. Martínez-Vicente, E. Perdiguero, et al., Autophagy maintains stemness by preventing senescence, *Nature* 529 (7584) (2016) 37–42, <https://doi.org/10.1038/nature16187>.
- [61] A.M. Joseph, P.J. Adhithetty, N.R. Wawrzyniak, et al., Dysregulation of mitochondrial quality control processes contribute to sarcopenia in a mouse model of premature aging, *PLoS One* 8 (7) (2013), e69327, <https://doi.org/10.1371/journal.pone.0069327>.
- [62] J.P. Leduc-Gaudet, O. Reynaud, S.N. Hussain, G. Gouspillou, Parkin overexpression protects from ageing-related loss of muscle mass and strength, *J. Physiol.* 597 (7) (2019) 1975–1991, <https://doi.org/10.1113/jp277157>.
- [63] D.W. Russ, A.M. Wills, I.M. Boyd, J. Krause, Weakness, SR function and stress in gastrocnemius muscles of aged male rats, *Exp. Gerontol.* 50 (2014) 40–44, <https://doi.org/10.1016/j.exger.2013.11.018>.
- [64] V.O. Shah, J. Scariano, D. Waters, et al., Mitochondrial DNA deletion and sarcopenia, *Genet. Med.* 11 (3) (2009) 147–152, <https://doi.org/10.1097/GIM.0b013e31819307a2>.
- [65] E. Doria, D. Buonocore, A. Focarelli, F. Marzatico, Relationship between human aging muscle and oxidative system pathway, *Oxidative Med. Cell. Longev.* 2012 (2012), 830257, <https://doi.org/10.1155/2012/830257>.
- [66] M.R. Beltran Valls, D.J. Wilkinson, M.V. Narici, et al., Protein carbonylation and heat shock proteins in human skeletal muscle: relationships to age and sarcopenia, *J. Gerontol. A Biol. Sci. Med. Sci.* 70 (2) (2015) 174–181, <https://doi.org/10.1093/gerona/glu007>.
- [67] N. Das, C.K. Jana, Age-associated oxidative modifications of mitochondrial α -subunit of F1 ATP synthase from mouse skeletal muscles, *Free Radic. Res.* 49 (8) (2015) 954–961, <https://doi.org/10.3109/10715762.2015.1017477>.
- [68] J. Feng, H. Xie, D.L. Meany, L.V. Thompson, E.A. Arriaga, T.J. Griffin, Quantitative proteomic profiling of muscle type-dependent and age-dependent protein carbonylation in rat skeletal muscle mitochondria, *J. Gerontol. A Biol. Sci. Med. Sci.* 63 (11) (2008) 1137–1152, <https://doi.org/10.1093/gerona/63.11.1137>.
- [69] E. Barreiro, C. Coronell, B. Laviña, A. Ramírez-Sarmiento, M. Orozco-Levi, J. Gea, Aging, sex differences, and oxidative stress in human respiratory and limb muscles, *Free Radic. Biol. Med.* 41 (5) (2006) 797–809, <https://doi.org/10.1016/j.freeradbiomed.2006.05.027>.
- [70] P. Gianni, K.J. Jan, M.J. Douglas, P.M. Stuart, M.A. Tarnopolsky, Oxidative stress and the mitochondrial theory of aging in human skeletal muscle, *Exp. Gerontol.* 39 (9) (2004) 1391–1400, <https://doi.org/10.1016/j.exger.2004.06.002>.
- [71] R.M. Alves, R. Vitorino, P. Figueiredo, J.A. Duarte, R. Ferreira, F. Amado, Lifelong physical activity modulation of the skeletal muscle mitochondrial proteome in mice, *J. Gerontol. A Biol. Sci. Med. Sci.* 65 (8) (2010) 832–842, <https://doi.org/10.1093/gerona/glq081>.
- [72] D. Buonocore, S. Rucci, M. Vandoni, M. Negro, F. Marzatico, Oxidative system in aged skeletal muscle, *Muscles Ligaments Tendons J.* 1 (3) (2011) 85–90.
- [73] O. Pansarasa, G. Felzani, J. Vecchiet, F. Marzatico, Antioxidant pathways in human aged skeletal muscle: relationship with the distribution of type II fibers, *Exp. Gerontol.* 37 (8–9) (2002) 1069–1075, [https://doi.org/10.1016/s0531-5565\(02\)00085-2](https://doi.org/10.1016/s0531-5565(02)00085-2).
- [74] M.S. Lustgarten, Y.C. Jang, Y. Liu, et al., MnSOD deficiency results in elevated oxidative stress and decreased mitochondrial function but does not lead to muscle atrophy during aging, *Aging Cell* 10 (3) (2011) 493–505, <https://doi.org/10.1111/j.1474-9726.2011.00695.x>.
- [75] G. Fanò, P. Mecocci, J. Vecchiet, et al., Age and sex influence on oxidative damage and functional status in human skeletal muscle, *J. Muscle Res. Cell Motil.* 22 (4) (2001) 345–351, <https://doi.org/10.1023/a:1013122805060>.
- [76] O. Pansarasa, L. Castagna, B. Colombi, J. Vecchiet, G. Felzani, F. Marzatico, Age and sex differences in human skeletal muscle: role of reactive oxygen species, *Free Radic. Res.* 33 (3) (2000) 287–293, <https://doi.org/10.1080/1071576000301451>.
- [77] M.J. Sullivan-Gunn, P.A. Lewandowski, Elevated hydrogen peroxide and decreased catalase and glutathione peroxidase protection are associated with aging sarcopenia, *BMC Geriatr.* 13 (2013) 104, <https://doi.org/10.1186/1471-2318-13-104>.
- [78] H. Xu, R. Ranjit, A. Richardson, H. Van Remmen, Muscle mitochondrial catalase expression prevents neuromuscular junction disruption, atrophy, and weakness in a mouse model of accelerated sarcopenia, *J. Cachexia. Sarcopenia Muscle* 12 (6) (2021) 1582–1596, <https://doi.org/10.1002/jcsm.12768>.
- [79] N.A. Foreman, A.S. Hesse, L.L. Ji, Redox signaling and sarcopenia: searching for the primary suspect, *Int. J. Mol. Sci.* 22 (16) (2021), <https://doi.org/10.3390/ijms22169045>.
- [80] K.-P. Lee, Y.J. Shin, S.C. Cho, et al., Peroxiredoxin 3 has a crucial role in the contractile function of skeletal muscle by regulating mitochondrial homeostasis, *Free Radic. Biol. Med.* 77 (2014) 298–306, <https://doi.org/10.1016/j.freeradbiomed.2014.09.010>.
- [81] T.S. Chang, C.S. Cho, S. Park, S. Yu, S.W. Kang, S.G. Rhee, Peroxiredoxin III, a mitochondrion-specific peroxidase, regulates apoptotic signaling by mitochondria, *J. Biol. Chem.* 279 (40) (2004) 41975–41984, <https://doi.org/10.1074/jbc.M407707200>.
- [82] B. Ahn, R. Ranjit, P. Kneis, et al., Scavenging mitochondrial hydrogen peroxide by peroxiredoxin 3 overexpression attenuates contractile dysfunction and muscle atrophy in a murine model of accelerated sarcopenia, *Aging Cell* 21 (3) (2022), e13569, <https://doi.org/10.1111/acel.13569>.
- [83] R. Ferreira, R. Vitorino, R.M. Alves, et al., Subsarcolemmal and intermyofibrillar mitochondria proteome differences disclose functional specializations in skeletal muscle, *Proteomics* 10 (17) (2010) 3142–3154, <https://doi.org/10.1002/pmic.201000173>.
- [84] M. Takahashi, D.A. Hood, Protein import into subsarcolemmal and intermyofibrillar skeletal muscle mitochondria. Differential import regulation in distinct subcellular regions, *J. Biol. Chem.* 271 (44) (1996) 27285–27291, <https://doi.org/10.1074/jbc.271.44.27285>.

- [85] V. Ljubicic, A.M. Joseph, P.J. Adhietty, et al., Molecular basis for an attenuated mitochondrial adaptive plasticity in aged skeletal muscle, *Aging* (Albany NY) 1 (9) (2009) 818–830, <https://doi.org/10.18632/aging.100083>.
- [86] J.P. Leduc-Gaudet, M. Picard, F. St-Jean Pelletier, et al., Mitochondrial morphology is altered in atrophied skeletal muscle of aged mice, *Oncotarget* 6 (20) (2015) 17923–17937, <https://doi.org/10.18632/oncotarget.4235>.
- [87] B. Chabi, V. Ljubicic, K.J. Menzies, J.H. Huang, A. Saleem, D.A. Hood, Mitochondrial function and apoptotic susceptibility in aging skeletal muscle, *Aging Cell* 7 (1) (2008) 2–12, <https://doi.org/10.1111/j.1474-9726.2007.00347.x>.
- [88] D.M. Callahan, N.G. Bedrin, M. Subramanian, et al., Age-related structural alterations in human skeletal muscle fibers and mitochondria are sex specific: relationship to single-fiber function, *J. Appl. Physiol.* 116 (12) (2014) 1582–1592, <https://doi.org/10.1152/jappphysiol.01362.2013>.
- [89] J.D. Crane, M.C. Devries, A. Safdar, M.J. Hamadeh, M.A. Tarnopolsky, The effect of aging on human skeletal muscle mitochondrial and intramyocellular lipid ultrastructure, *J. Gerontol. A Biol. Sci. Med. Sci.* 65 (2) (2010) 119–128, <https://doi.org/10.1093/geron/glp179>.
- [90] B. Colom, M.P. Alcolea, A. Valle, J. Oliver, P. Roca, F.J. García-Palmer, Skeletal muscle of female rats exhibit higher mitochondrial mass and oxidative-phosphorylative capacities compared to males, *Cell. Physiol. Biochem.* 19 (1–4) (2007) 205–212, <https://doi.org/10.1159/000099208>.
- [91] J. Viña, C. Borrás, J. Gambini, J. Sastre, F.V. Pallardó, Why females live longer than males? Importance of the upregulation of longevity-associated genes by oestrogenic compounds, *FEBS Lett.* 579 (12) (2005) 2541–2545, <https://doi.org/10.1016/j.febslet.2005.03.090>.
- [92] L.A. Gómez, J.S. Monette, J.D. Chavez, C.S. Maier, T.M. Hagen, Supercomplexes of the mitochondrial electron transport chain decline in the aging rat heart, *Arch. Biochem. Biophys.* 490 (1) (2009) 30–35, <https://doi.org/10.1016/j.abb.2009.08.002>.
- [93] S. Kumaran, M. Subathra, M. Balu, C. Panneerselvam, Age-associated decreased activities of mitochondrial electron transport chain complexes in heart and skeletal muscle: role of L-carnitine, *Chem. Biol. Interact.* 148 (1–2) (2004) 11–18, <https://doi.org/10.1016/j.cbi.2003.10.010>.
- [94] M. Triolo, A.N. Oliveira, R. Kumari, D.A. Hood, The influence of age, sex, and exercise on autophagy, mitophagy, and lysosome biogenesis in skeletal muscle, *Skelet. Muscle* 12 (1) (2022) 13, <https://doi.org/10.1186/s13395-022-00296-7>.
- [95] X. Tian, S. Lou, R. Shi, From mitochondria to sarcopenia: role of 17 β -estradiol and testosterone, *Front. Endocrinol. (Lausanne)* 14 (2023) 1156583, <https://doi.org/10.3389/fendo.2023.1156583>.
- [96] A. Vasconsuelo, L. Milanese, R. Boland, Actions of 17 β -estradiol and testosterone in the mitochondria and their implications in aging, *Ageing Res. Rev.* 12 (4) (2013) 907–917, <https://doi.org/10.1016/j.arr.2013.09.001>.
- [97] L. Milanese, A. Russo de Boland, R. Boland, Expression and localization of estrogen receptor alpha in the C2C12 murine skeletal muscle cell line, *J. Cell. Biochem.* 104 (4) (2008) 1254–1273, <https://doi.org/10.1002/jcb.21706>.
- [98] L. Milanese, A. Vasconsuelo, A.R. de Boland, R. Boland, Expression and subcellular distribution of native estrogen receptor beta in murine C2C12 cells and skeletal muscle tissue, *Steroids* 74 (6) (2009) 489–497, <https://doi.org/10.1016/j.steroids.2009.01.005>.
- [99] L. Pronsato, R. Boland, L. Milanese, Non-classical localization of androgen receptor in the C2C12 skeletal muscle cell line, *Arch. Biochem. Biophys.* 530 (1) (2013) 13–22, <https://doi.org/10.1016/j.abb.2012.12.011>.
- [100] S.H. Yang, R. Liu, E.J. Perez, et al., Mitochondrial localization of estrogen receptor beta, *Proc. Natl. Acad. Sci. U. S. A.* 101 (12) (2004) 4130–4135, <https://doi.org/10.1073/pnas.0306948101>.
- [101] D. Montero, K. Madsen, A.-K. Meinild-Lundby, F. Edin, C. Lundby, Sexual dimorphism of substrate utilization: differences in skeletal muscle mitochondrial volume density and function, *Exp. Physiol.* 103 (6) (2018) 851–859, <https://doi.org/10.1113/EP087007>.
- [102] D. Watanabe, K. Hatakeyama, R. Ikegami, et al., Sex differences in mitochondrial Ca(2+) handling in mouse fast-twitch skeletal muscle in vivo, *J. Appl. Physiol.* 128 (2) (2020) 241–251, <https://doi.org/10.1152/jappphysiol.00230.2019>.
- [103] M.E. Lindholm, M. Huss, B.W. Solnestam, S. Kjellqvist, J. Lundberg, C. J. Sundberg, The human skeletal muscle transcriptome: sex differences, alternative splicing, and tissue homogeneity assessed with RNA sequencing, *FASEB J.* 28 (10) (2014) 4571–4581, <https://doi.org/10.1096/fj.14-255000>.
- [104] J.Q. Chen, P.R. Cammarata, C.P. Baines, J.D. Yager, Regulation of mitochondrial respiratory chain biogenesis by estrogens/estrogen receptors and physiological, pathological and pharmacological implications, *Biochim. Biophys. Acta* 1793 (10) (2009) 1540–1570, <https://doi.org/10.1016/j.bbamcr.2009.06.001>.
- [105] R.C. Scarpulla, R.B. Vega, D.P. Kelly, Transcriptional integration of mitochondrial biogenesis, *Trends Endocrinol. Metab.* 23 (9) (2012) 459–466, <https://doi.org/10.1016/j.tem.2012.06.006>.
- [106] V. Ribas, B.G. Drew, Z. Zhou, et al., Skeletal muscle action of estrogen receptor α is critical for the maintenance of mitochondrial function and metabolic homeostasis in females, *Sci. Transl. Med.* 8 (334) (2016), 334ra354, <https://doi.org/10.1126/scitranslmed.aad3815>.
- [107] A. Vasconsuelo, L. Milanese, R. Boland, Participation of HSP27 in the antiapoptotic action of 17 β -estradiol in skeletal muscle cells, *Cell Stress Chaperones* 15 (2) (2010) 183–192, <https://doi.org/10.1007/s12192-009-0132-y>.
- [108] M.J. Torres, K.A. Kew, T.E. Ryan, et al., 17 β -estradiol directly lowers mitochondrial membrane microviscosity and improves bioenergetic function in skeletal muscle, *Cell Metab.* 27 (1) (2018) 167–179.e167, <https://doi.org/10.1016/j.cmet.2017.10.003>.
- [109] W. Guo, S. Wong, M. Li, et al., Testosterone plus low-intensity physical training in late life improves functional performance, skeletal muscle mitochondrial biogenesis, and mitochondrial quality control in male mice, *PLoS One* 7 (12) (2012), e51180, <https://doi.org/10.1371/journal.pone.0051180>.
- [110] T. Usui, K. Kajita, T. Kajita, et al., Elevated mitochondrial biogenesis in skeletal muscle is associated with testosterone-induced body weight loss in male mice, *FEBS Lett.* 588 (10) (2014) 1935–1941, <https://doi.org/10.1016/j.febslet.2014.03.051>.
- [111] D.A. Cardinale, O. Horwath, J. Elings-Knutsson, et al., Enhanced skeletal muscle oxidative capacity and capillary-to-fiber ratio following moderately increased testosterone exposure in young healthy women, *Front. Physiol.* 11 (2020), 585490, <https://doi.org/10.3389/fphys.2020.585490>.