

Review

OPA1 regulation of mitochondrial dynamics in skeletal and cardiac muscle

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The mitochondria are double-membrane organelles integral for energy metabolism. Mitochondrial dynamics is regulated by inner and outer mitochondrial membrane (IMM and OMM) proteins, which promote fission and fusion. Optic atrophy 1 (OPA1) regulates IMM fusion, prevents apoptosis, and is a key regulator of morphological change in skeletal and cardiac muscle physiology and pathophysiology. OPA1 fuses the inner membranes of adjacent mitochondria, allowing for an increase in oxidative phosphorylation (OXPHOS). Considering the importance of energy metabolism in whole-body physiology, OPA1 and its regulators have been proposed as novel targets for the treatment of skeletal muscle atrophy and heart failure. Here, we review the role and regulation of OPA1 in skeletal muscle and cardiac pathophysiology, epitomizing its critical role in the cell.

Mitochondrial dynamics and the regulation of mitochondrial function

Mitochondria are known for their role in energy metabolism and play important roles in regulating apoptosis, calcium storage, and signaling. Mitochondria are extremely dynamic organelles that undergo continuous morphological changes through the processes of mitochondrial biogenesis, mitochondrial fusion–fission dynamics, and mitochondrial mitophagy, assisting in the formation of a complex mitochondrial reticulum (the mitochondrial life cycle, as outlined in Figure 1). Mitochondrial quality control mechanisms maintain mitochondrial integrity and function when subject to stress such as exercise or inactivity. Mitochondrial function is largely dependent on the number and size of the organelles, which is determined by the balance between these highly conserved, innate processes. The network of mitochondrial organelles is well adapted to meet the energy demands of the cell, and dysregulation of these processes has been proposed to be integral to pathological states, particularly in skeletal and cardiac muscle.

Mitochondrial fission

Mitochondrial morphology, which varies across cell type, is regulated by the balance of mitochondrial fusion and fission [1]. As outlined in Figure 2, mitochondrial fission is the division of larger mitochondrial reticula into smaller mitochondrial reticula and is regulated by dynamin-related protein 1 (Drp1), a GTP hydrolyzing enzyme, along with fission protein 1 (Fis1) and mitochondrial fission factor (Mff). Other regulators of mitochondrial fission have also been identified, such as mitochondrial dynamics proteins of 49 and 51 kDa (MiD49 and MiD51); however, these have also been noted to play a regulatory role in mitochondrial fusion by inhibiting Drp1 GTPase activity, as emphasized by its alias name: mitochondrial elongation factors 1/2 (MIEF 1/2) [2]. Mitochondrial fission is initiated by the recruitment of the cytosolic Drp1 to the OMM, where it binds and forms spiral structures on the surface of the mitochondrial membrane. Such spiraling is the initial step in mitochondrial constriction and subsequent abscission [3]. As mitochondria undergo fission and fusion, they create connections with other organelles within the cell, such as the endoplasmic reticulum (ER), lysosomes, and peroxisomes [4]. These direct interorganelle interactions, often referred to

Highlights

Mitochondria are known for their role in energy metabolism and play important roles in regulating apoptosis, calcium storage, and signaling.

Optic atrophy 1 (OPA1) mediates inner mitochondrial membranes of adjacent mitochondria and is therefore crucial in increasing mitochondrial membrane cross-sectional area and thus oxidative phosphorylation.

A reduction in OPA1 expression leads to mitochondrial fragmentation, resulting in skeletal muscle atrophy, and has been linked to the onset of metabolic disease such as type 2 diabetes.

Altered OPA1 function has been proposed to contribute to the pathogenesis of heart failure.

Chronic/acute exercise training results in more fused, tubular skeletal muscle mitochondrial networks, which is reflected by an increase in skeletal muscle OPA1 expression.

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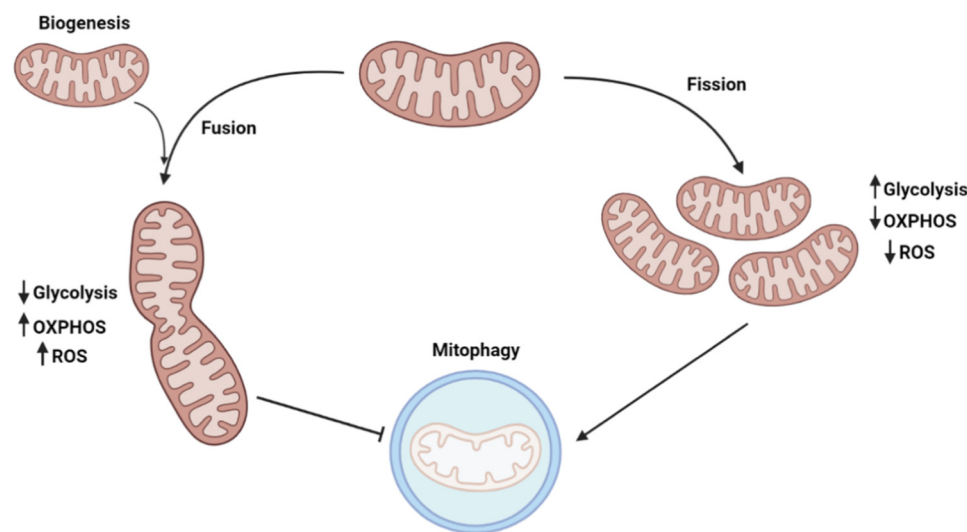
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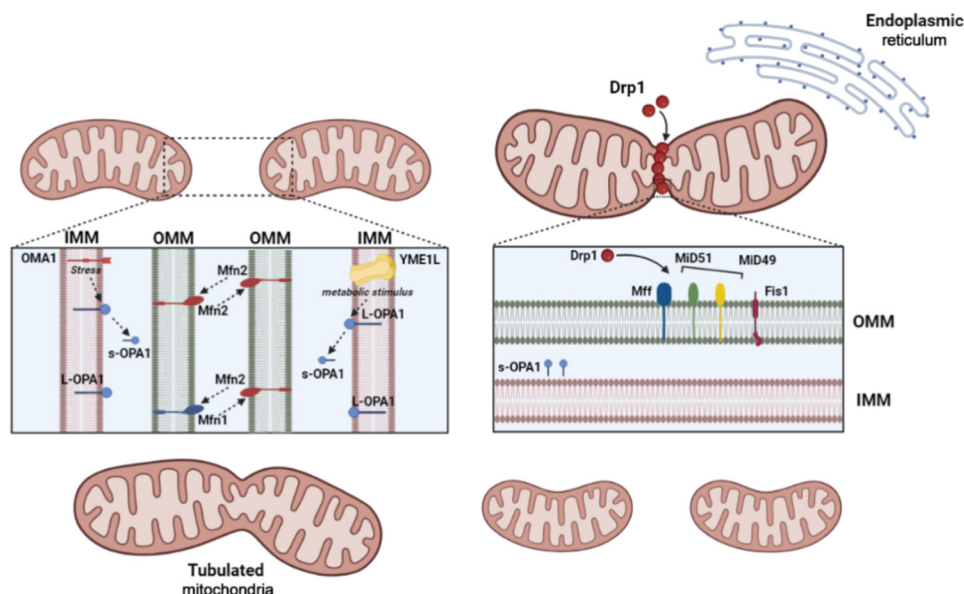
Figure 1. The mitochondrial life cycle. The mitochondria undergo a process of changing and remodeling by the integrated balance of mitochondrial biogenesis, morphology, and mitophagy impacting upon glycolysis, oxidative phosphorylation (OXPHOS), and reactive oxygen species (ROS).

as membrane contact sites, are considered the most effective way for mitochondria and other organelles to communicate with each other, improving signal transmission, material exchange (i.e., calcium), and cell maintenance and playing a key role in mitochondrial dynamics [5]. In particular, mitochondrial-ER contact sites have also been shown to determine the site of mitochondrial fission. Live cell imaging studies show how the ER may act to wrap around the mitochondria at the site of mitochondrial fission, known as constriction sites [6]. Mff, MiD49, MiD51, and Drp1 are known to colocalize at these constriction sites, although, these sites have also been shown to constrict in the absence of Mff and Drp1 [6].

Mitochondrial fusion

Mitochondrial fusion is orchestrated by both IMM and OMM proteins, which communicate with each other to join adjacent mitochondria together and increase mitochondrial cross-sectional area (CSA) [7,8]. OMM fusion is regulated by mitofusin 1 and 2 (Mfn1 and Mfn2), along with MiD49 and MiD51. They form homo- or heterotypic complexes imperative for the two-step process of mitochondrial fusion, initiated by the tethering of two adjacent mitochondria on their membranes by specific Mfn1/Mfn2 domains followed by the GTPase docking of both membranes prior to final fusion. Fusion of the IMM is regulated by OPA1 [9], and it remains to be determined if these two processes are coupled.

Independent of its role in IMM fusion, OPA1 also plays a role in cristae remodeling and has been considered an integral regulator of OXPHOS. Cristae membranes are highly dynamic and continuously undergo morphological changes mediated by OPA1, the i-AAA protease, ATP-dependent zinc metalloprotease 1 (YME1L), metalloendopeptidase (OMA1), mitochondrial contact site and cristae organizing system (MICOS), and sorting and assembly machinery complex 50 (Sam50) [10]. Fusion events can be extremely brief, exchanging contents but having no morphological impact. Such events are referred to as so-called 'kiss and run' fusion events, acting as a rapid re-charge mechanism providing a means to rescue the function of mitochondrial organelles void of some key components [11].



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Figure 2. Regulation of mitochondrial fusion and fission. Mitochondrial fusion (left) is regulated by inner and outer mitochondrial membrane (IMM and OMM) fusion targets. OMM fusion is mediated by interactions between mitofusin 1 (Mfn1) (dark blue) and mitofusin 2 (Mfn2) (red), while IMM fusion is mediated by homo- and heterotypic interactions between long-optic atrophy 1 (L-OPA1) (blue). L-OPA1 and short-optic atrophy 1 (S-OPA1) expression and activity are regulated by i-AAA protease, ATP-dependent zinc metalloprotease 1 (YME1L) (yellow), at times of metabolic change and metalloendopeptidase (OMA1) (red) during times of stress. Mitochondrial fission (right) is regulated following the recruitment of cytosolic dynamin-related protein 1 (Drp1) (red) to the OMM by fission protein 1 (Fis1) (red), mitochondrial fission factor (Mff) (purple), mitochondrial dynamics protein 49 (MID49) (yellow), and/or mitochondrial dynamics protein 51 (MID51) (green) to locations of division marked by the endoplasmic reticulum (ER, blue).

Mitochondrial fusion optimizes maximal oxidative capacity and is therefore a vital process for overall cellular metabolism. Mitochondrial fusion also mitigates cellular stress by mixing the contents of partially damaged mitochondria [12]. In addition to its essential role in energy provision, fusion between mitochondria has also been proposed to rescue mitochondria with mutations (as long as the mutation load remains below 80–90% per cell) by cross-complementation, mitigating the effects of environmental damage by the exchange of proteins, lipids, and mitochondrial DNA (mtDNA) between mitochondria [12,13]. This is supported by Yang *et al.* [14], who report that a small proportion of mitochondria from the Rho0 HeLa cell line, lacking mtDNA, can have their function restored by fusing with normal mitochondria (“initial metabolic complementation”), maintaining mitochondrial genome integrity.

This mitochondrial component sharing is thought to be a compensatory mechanism to maintain cellular homeostasis [12]. Civiletto *et al.* [15], demonstrated an improvement in respiratory chain efficiency with OPA1 overexpression in two mitochondrial disease mouse models (offspring from the crosses of a constitutive knockout for structural complex I component Ndufs4 and muscle-specific conditional knockout for complex IV assembly factor Cox15, with Opa1 transgenic mice). This literature suggested that overexpression resulted in an improved lifespan (62.0 vs. 53.5 days), a 1.5-fold increase in state 3 oxygen consumption, and a doubling of the average gastrocnemius skeletal muscle fiber CSA relative to the naive non-Opa1-overexpressing models. For this reason, mitochondrial fusion has been proposed to have a multifaceted role, from maintaining a mitochondrial reticulum to cellular homeostasis.

Maintenance of mitochondrial dynamics is essential for optimal cellular function. This is especially important in the heart, where the mitochondria occupy 30% the total cell volume [16]. Skeletal muscle mitochondria are also highly dynamic, and mitochondrial morphological changes are observed in response to exercise [17] and aging [18]. In response to environmental stress, mitochondria are constantly changing their shape through fusion and fission events, with the net balance between fusion and fission controlling the shape and number of mitochondria.

Given the importance of mitochondrial dynamics for optimal cell function, the IMM fusion protein, OPA1, has emerged as an integral player in the regulation of mitochondrial function, particularly in relation to skeletal and cardiac muscle physiology.

Function and regulation of OPA1

OPA1 is a dynamin-related GTPase located in the IMM and regulates inner membrane fusion. Due to alternative splicing and cleavage, OPA1 gives rise to eight human isoforms, a long form (L-OPA1), which is proteolytically cleaved to short forms (S-OPA1). L-OPA1 is anchored to the IMM, whereas S-OPA1 is enriched in the intermembrane space (IMS). Both L-OPA1 and S-OPA1 are processed and maintained in balance by the actions of OMA1 and YME1L, which act to cleave the IMM-bound L-OPA1 to the soluble S-OPA1. The proteolytic actions of OMA1 appear to occur in times of stress (stress-induced fission), while the actions of YME1L are a response to metabolic stimuli [4]. A predominance of L-OPA1 leads to mitochondrial fusion, but an increase in the activity of OMA1 and YME1L initiates L-OPA1 proteolysis, resulting in a shift to more S-OPA1 and overall mitochondrial fission [19]. Fusion subsequently occurs once Mfn1/2 fuse to the OMM of adjacent mitochondria and the L-OPA1, which tethers to the inner membrane space and, facing the IMS, binds to another L-OPA1 on the IMM of the adjacent mitochondrion [20].

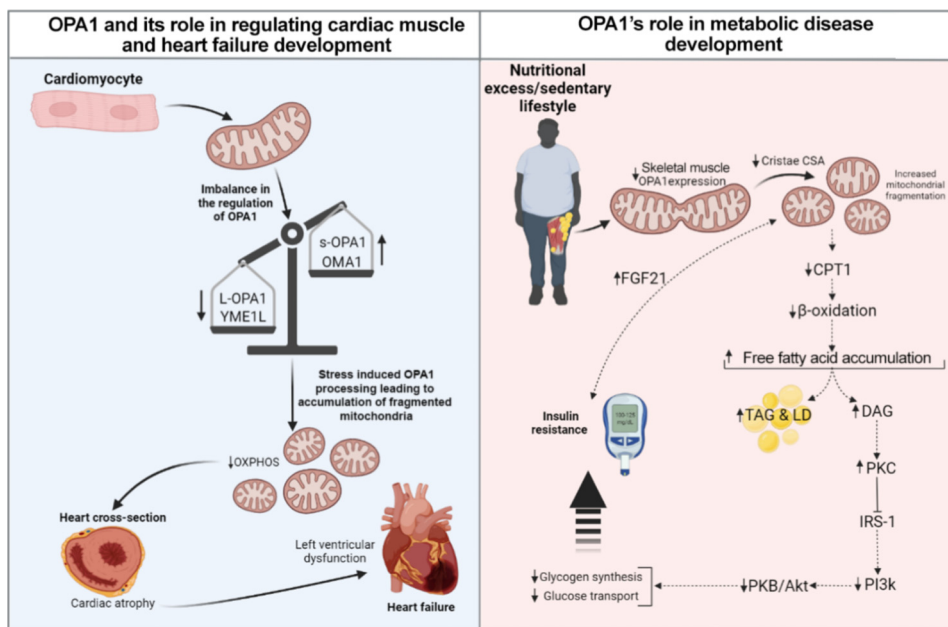
In addition to its role in mitochondrial fusion, OPA1 is implicated in mtDNA maintenance [21], the prevention of apoptosis [22], as well as mitochondrial respiration and super complex assembly. OPA1 is also crucial for the maintenance and organization of cristae structure [23] and more recently has been associated with the regulation of mitochondrial calcium. Fülöp *et al.* [24] reported that the *in vitro* knockdown of OPA1 in HeLa increased mitochondrial calcium uptake by increasing the access of calcium to its transporters in the cristae membrane. Emerging evidence suggests that abnormal calcium homeostasis contributes to the neurodegenerative disease dominant optic atrophy (DOA), resulting from a mutation in OPA1, known to lead to blindness [25].

Function and regulation of OPA1 in cardiac muscle and heart failure

Heart failure is a life-threatening disease affecting 26 million people worldwide [26], highlighting the need for new and novel treatment strategies. With heart failure, cardiomyocytes undergo complex changes that lead to contractile dysfunction. Altered mitochondrial dynamics may contribute to the pathogenesis of heart failure [27] as imbalanced mitochondrial dynamics leads to apoptosis, mitochondrial mutations, and reduced respiratory capacity [28]. Optimal cardiomyocyte function requires a dynamic balance between fission and fusion of the mitochondrial network [29] to meet the metabolic demands of the heart. OPA1 protein has been shown to be reduced in both human and rat models of heart failure [30], highlighting the potential role of OPA1 in altered mitochondrial dynamics associated with heart failure. In addition to its role in mitochondrial fusion, OPA1 also plays a significant role in maintaining cristae morphology and protecting against the release of cytochrome c and the onset of apoptosis [22,30].

OPA1 ablation resulted in small and fragmented mitochondria consistent with impaired fusion [30]. As complete ablation of OPA1 is embryonically lethal [31], heterozygous (OPA1^{+/-})

mice have been used to study the role of OPA1 in the heart. OPA1^{+/-} have displayed late-onset (12 months) left ventricular dysfunction associated with impaired mitochondrial function [32] and vision impairment, characteristic of DOA. Germline OPA1 heterozygous mice exhibit reduced mtDNA, a twofold increase in cardiac hypertrophy after transaortic constriction, altered cristae morphology, but preserved mitochondrial bioenergetics [33]. Additionally, mitochondrial permeability transition pore opening was less sensitive to calcium accumulation in OPA1 heterozygous mice [33]. Using a conditional mouse model for OMA1 and YME1L, the two known proteases of OPA1, Wai *et al.* [34] examined the role of OPA1 processing in the development of heart failure. As illustrated in Figure 3 (left, blue), loss of YME1L, and therefore L-OPA1 forms and activation of OMA1, and the accumulation of S-OPA1 triggered stress-induced OPA1 processing, resulting in the accumulation of small, fragmented mitochondria in the heart. However, deletion of OMA1 has been observed to restore mitochondrial morphology in cardiomyocytes lacking YME1L [34].



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Figure 3. The role of OPA1 in heart failure and metabolic disease development. Optic atrophy 1 (OPA1) regulates cardiac muscle and heart failure (left, blue). An imbalance in OPA1 regulation resulting from a decrease in i-AAA protease, ATP-dependent zinc metalloprotease 1 (YME1L), leads to a decrease in long-OPA1 (L-OPA1), an increase in metalloendopeptidase (OMA1), and short-OPA1 (S-OPA1) expression. Such changes lead to stress-induced OPA1 processing, leading to mitochondrial fragmentation. A decrease in mitochondrial size leads to a reduction in cristae cross-sectional area (CSA), leading to a decrease in oxidative phosphorylation (OXPHOS). Such reduction leads to an increase in cardiac atrophy resulting from left ventricular dysfunction, leading to heart failure. OPA1 plays a significant role in metabolic disease development (right, red). Nutritional excess/sedentary lifestyle decreases skeletal muscle OPA1 expression, decreasing cristae CSA, resulting in an increase in mitochondrial fragmentation. An increase in fragmentation decreases OXPHOS and, subsequently, the capacity of the mitochondria to oxidize lipid species. A decrease in OXPHOS has been shown to result in an accumulation of adverse lipid species such as triacylglycerides (TAGs) and diacylglycerides (DAGs), subsequently increasing lipid droplet (LD) size. An increase in DAG has been observed to impact the insulin signaling cascade by initially increasing protein kinase C (PKC), which acts to inhibit the activity of insulin receptor substrate-1 (IRS-1). This leads to a decrease in phosphoinositide 3-kinase (PI3K), which results in a decrease in protein kinase B (PKB/Akt) linked to a decrease in glycogen synthesis and glucose transport within the skeletal muscle. This process is covered in-depth in a review by Chavez and Summers [45]. Such adverse changes to glucose metabolism have been shown to lead to an increase in insulin resistance, which can lead to the development of type 2 diabetes (T2DM). An increase in fibroblast growth factor 21 (FGF21) has been observed to be significantly increased in those with insulin resistance and with OPA1 inhibition.

Altered mitochondrial metabolism is the underlying basis for increased sensitivity to stress in an aging heart, resulting in respiratory defects. The complexes of the electron transport chain are organized in supercomplexes, which are altered in the aged heart [35]. These changes have been observed after the onset of heart failure [36]. Furthermore, abnormalities in mitochondrial dynamics contribute to the aging process [12]. As defective mitochondria are associated with enhanced oxidant production in aging, the regulation of OPA1 in mitochondrial dynamics in cardiac aging could represent a potential therapeutic target.

Impaired myocardial insulin signaling could precipitate mitochondrial dysfunction in the heart [37], while insulin has been shown to stimulate mitochondrial fusion and function in cardiomyocytes via an Akt-mTOR-NFκB-OPA1 mediated signaling pathway, signifying a novel link between mitochondrial morphology, OPA1, and insulin signaling [38]. Furthermore, cardiac lipid overload altered OPA1 processing by increasing reactive oxygen species production [39]. These findings suggest an important role for OPA1 in cardiac physiology and implicate OPA1 in cardiac pathophysiology. Future studies will determine the mechanism by which OPA1 plays a role in heart failure development.

Altered role of OPA1 in metabolic disease development

Metabolic diseases such as obesity and type 2 diabetes (T2DM) have increased dramatically in recent years. According to the non-communicable disease risk factor collaboration, the number of adults with obesity increased ~570% worldwide between 1975 and 2016 (100–671 million) [40], with at least 4 million deaths directly linked to the disease in 2021 according to WHO. WHO also calculated that in 2021, there was a total of 463 million people living with diabetes, a disease with an annual rate of all-cause mortality averaging 23 per 1000 people [41].

Given the burden of metabolic diseases, there is a need to better understand the underpinning mechanisms to develop alternative therapeutic strategies. Considering the link between metabolic disease and the capacity of the mitochondria to select fuel in response to nutritional change [42], dysfunction to mitochondrial dynamics has been proposed to be integral in the pathophysiology of metabolic disease progression [43]. As OPA1 plays a central role in the regulation of mitochondrial morphology, energy metabolism, calcium regulation, and apoptosis, it could be a potential therapeutic target for the treatment of metabolic disease.

Elongated mitochondria have been identified to possess an increased capacity to generate energy through OXPHOS, preferentially oxidizing lipid substrates through β-oxidation over glycolysis [44]. OPA1, through its promotion of mitochondrial fusion, increases cristae CSA, allowing for greater distribution of OXPHOS proteins in the cristae, resulting in a greater capacity for energy production [7,8].

Nutritional excess is one of the main contributors to increased lipid accumulation in tissues, particularly skeletal muscle, and is associated with a significant reduction in mitochondrial oxidative capacity, leading to insulin resistance via its role in the protein kinase B (PKB/Akt) insulin signaling cascade (as covered in-depth in a review by Chavez and Summers, [45]), T2DM, and obesity (Figure 3, right, red) [46]. In addition to a reduction in mitochondrial function with obesity-related nutrient overload, a fragmented mitochondrial network has also been observed under these same conditions, aiding in the progression of metabolic disease [47,48]. Conversely, during mild fasting conditions in culture (mouse embryonic fibroblasts), mitochondria appear more interconnected [42], supporting the notion that mitochondrial elongation is an active mechanism aiding to increase mitochondrial bioenergetic efficiency.

Kelley *et al.* [47] were one of the first groups to recognize that mitochondrial area was significantly decreased (a 35% reduction) as a result of diet-induced obesity. Since then, evidence has

emerged that obesity could be inducing mitochondrial fragmentation in skeletal muscle, as it is correlated with a decrease in insulin sensitivity and is associated with the previously observed reduction in mitochondrial respiratory capacity. OPA1 and Fis1 protein expression is significantly decreased in obese individuals compared with lean, healthy individuals [48]. As signified in Figure 3 (right, red), the decreased expression of OPA1 could explain these changes, reducing cristae CSA, decreasing the expression of CPT1, subsequently decreasing β -oxidation, leading to a paradoxical accumulation of free fatty acids, which have been shown to progress metabolic disease development resulting in insulin resistance. Whereas the decrease in Fis1 expression could be indicative of decreased mitochondrial fission, reduced mitochondrial quality control, and an accumulation of dysfunctional mitochondria, given the interrelationship between dynamics and mitophagy/mitochondrial turnover [12]. In a genetic model of obesity, obese male Zucker rats had decreased Mfn2 expression [49], supporting the role of altered fusion dynamics and mitochondrial fragmentation. Houzelle *et al.* [48] confirmed this observation and found it was concomitant with significant alteration to the mitochondrial network and a reduction in OXPHOS.

Therapeutic potential of OPA1

Skeletal and cardiac muscle knockout and overexpression studies have given novel insight into the crucial role OPA1 plays in mitochondrial bioenergetics, mitochondrial networks, and whole-body metabolism, but has also signified the great potential such focus may have in the therapy of metabolic disease development. Romanello *et al.* [50] reported that whole-body OPA1 knockout in adult mice presented a phenotype of sarcopenia and unhealthy aging, ultimately leading to premature death and excessive mitochondrial fragmentation. However, with specific skeletal muscle OPA1 inhibition, less severe adaptations are evident, as supported by an integrated stress response culminated by an observed increase in insulin sensitivity and metabolic rate, changes attributed to inflammation and activating transcription factor 4 (ATF4), which has been reported to regulate moderate secretion of the stress-response myokine, fibroblast growth factor 21 (FGF21), from the skeletal muscle [42,51–53]. Although it is still heavily debated [54], a moderate induction of FGF21 results in an adaptive response, as can be seen with mild OPA1 inhibition, but a substantial increase has proven maladaptive and is associated with metabolic disease progression, such as T2DM and increased mortality [52,55]. As such, although there are clear (in) direct regulators of OPA1, FGF21 may be a novel target.

Conversely, OPA1 overexpression studies suggest that OPA1 has a protective effect from acute muscle atrophy induced by denervation in mice [56]. It has been suggested that this is due to its ability to increase cristae CSA, leading to an increased capacity for OXPHOS.

Subsequently, the results from these OPA1 knockdown/overexpression studies in *in vitro* cell culture and *in vivo* animal models have placed OPA1 as a novel target for the effective therapy of primary mitochondrial disorders in humans [15]. An example of such conditions is autosomal DOA (ADOA), a syndrome with no known cure and one of the most common inherited optic neuropathies caused by mutations in the nuclear OPA1 gene located on chromosome 3q28-q29 [57]. Mutation in OPA1 results in a ~50% reduction in OPA1 expression, supporting haploinsufficiency as a major disease mechanism in ADOA, ultimately impairing mitochondrial function and disorganizing mitochondria cristae structures [58]. It is a rare eye disorder causing vision and hearing loss but can also result in muscle weakness (myopathy), ataxia, and peripheral neuropathy. Advancements of therapies for mutation in OPA1 expression are in their infancy at present. Although studies focused on OPA1 mutation, correction through CRISPR-Cas9-guided homology-directed repair *in vitro* has shown potential by re-establishing mitochondrial homeostasis and its well-defined reticulum, as emphasized by Sladen *et al.* [59]. OPA1 protein expression has been shown to be reduced in human and rat models of heart failure, placing OPA1 as a

potentially important mediator of heart failure development; no published evidence to date demonstrates that ADOA is directly linked to heart failure.

Given the evidence linking OPA1 to disease progression, further research targeting OPA1 both pharmacologically and genetically could prove to have novel outcomes for such conditions.

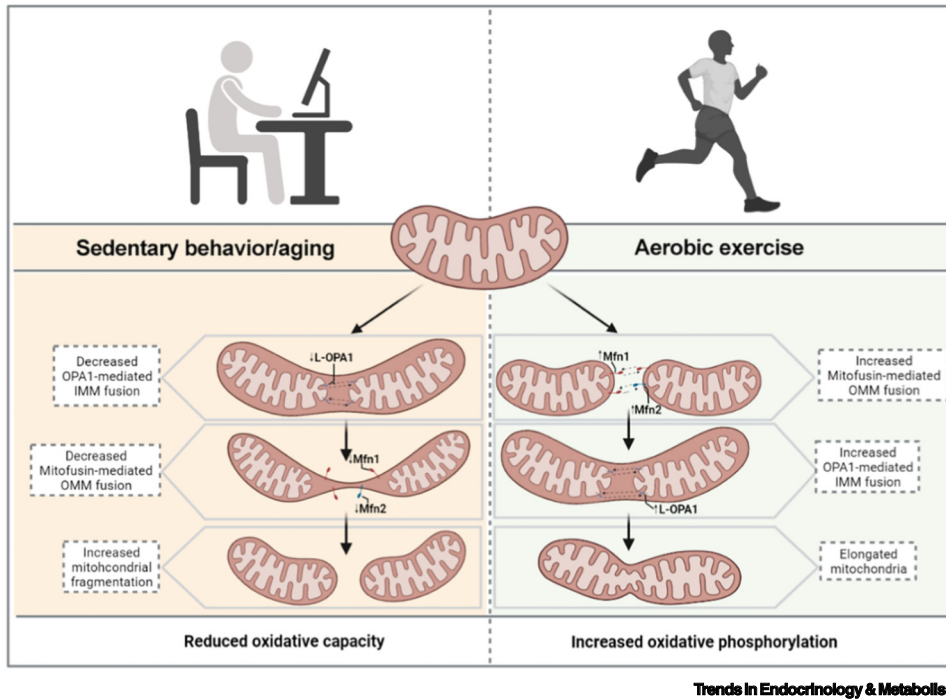
Function and regulation of OPA1 with physical inactivity and aging

Skeletal muscle mass begins to decline during the third and fourth decade of life, with approximately 10% of muscle lost by 50 years of age [60], with further decreases of 0.7–0.8% per year by 70 years of age [61]. Physical inactivity has similar effects on muscle mass, leading to reductions in muscle fiber number and CSA [62]. Short-term models of extreme physical inactivity, such as bed rest in humans [63] or hindlimb suspension in animals [64], are often used to study muscle mass regulation or ‘secondary aging’ [65]. Physical inactivity negatively impacts metabolic function in healthy young and older males. This is supported by a reduction in resting metabolic rate, insulin sensitivity, and mitochondrial respiration when subjected to 10–21 days of bed rest [66–68].

The induction of muscle atrophy with physical inactivity is associated with an imbalance in mitochondrial dynamics [69]. Sedentary individuals, when compared with age-matched, exercising counterparts, had a 50% reduction in the expression of mitochondrial fusion transcripts, OPA1, Mfn1, and Mfn2, and the fission transcript, Drp1, suggesting that exercise acts to maintain or prevent the decline in the expression of these regulators. Tezze *et al.* [52] explored if regulators of mitochondrial dynamics correlated with age-related muscle loss and, as highlighted in Figure 4, found a significant positive correlation with OPA1, but not Mfn1 or Drp1. Furthermore, mice subjected to 3 days of hindlimb suspension have a reduction in mitochondrial function, accompanied by a decrease in skeletal muscle OPA1, Mfn1, and Mfn2 transcripts but no change in Drp1 protein content. However, in the same study, they found after 7 days of hindlimb suspension, only Mfn1 and OPA1 mRNA was reduced. These transcriptional changes were in line with mitochondrial function and Drp1 protein expression, which subsequently decreased as hindlimb suspension progressed [70]. This delay in adaptability of regulators of fission in comparison with fusion with reduced activity emphasizes the sensitivity and flexibility of regulators of mitochondrial fusion to environmental stress. It remains unknown if OPA1 plays a direct causative role in physical inactivity-induced muscle atrophy; changes in OPA1 transcriptional activity and protein expression would suggest that OPA1 is altered with physical inactivity, suggesting it is an important component in the mitochondrial response to physical inactivity.

Function and regulation of OPA1 with exercise

Exercise has been well established as an effective countermeasure to prevent a multitude of adverse changes associated with physical inactivity and aging. Acute exercise and exercise training regimes have been shown to have significant and sometimes opposing effects on markers of mitochondrial dynamics. Aerobic exercise training has been shown to increase the translocation of the ‘master regulator’ of mitochondrial biogenesis, PGC-1 α , to the mitochondria and the nuclei, where it functions as a coactivator for both mitochondrial and nuclear transcription factors [71]. In addition to its role in regulating mitochondrial number, PGC-1 α has been shown to regulate mitochondrial dynamics by coactivating the expression of OMM fusion targets Mfn1 and Mfn2. Additionally, work by Caffin *et al.* [72] further emphasized the important role PGC-1 α , and mitochondrial biogenesis, has on IMM fusion target, OPA1 and vice versa. This work concluded that *Opa1*^{+/-} mice had a defect in mitochondrial biogenesis, an alteration that can have many adverse effects, including changes to substrate oxidation. Another thought-provoking change this literature evoked was that the impairment in biogenesis at rest was compensated during exercise



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Figure 4. Mitochondrial dynamic changes in aging and aerobic exercise. Aging and exercise have two opposing effects on mitochondrial dynamics, although their regulation is different. Sedentary behavior (left, orange) results in inner mitochondrial membrane (IMM) fragmentation instigated by a decrease in long-optic atrophy 1 (L-OPA1) expression. This reduction in L-OPA1 initiates a reduction in IMM cross-sectional area (CSA) due to the imbalance between fusion and fission in favor of fission. With continued sedentary behavior or with aging outer mitochondrial fusion regulators along the outer mitochondrial membrane (OMM), mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2), decrease in expression, resulting in an imbalance in fusion–fission weighted towards fission/fragmentation. Such behavior results in a decrease in mitochondrial oxidative capacity. Aerobic exercise (right, green) leads to an increase in mitochondrial size, increasing overall oxidative phosphorylation. Aerobic exercise increases the expression of outer mitochondrial fusion regulators, Mfn1 and Mfn2, allowing for greater binding of mitochondrial reticula, together increasing the OMM CSA. Such an increase in OMM CSA is then followed by an increase in L-OPA1 expression, which acts to increase IMM CSA.

by an apparent indirect activation of IMM fatty acid transporter, carnitine palmitoyltransferase 2 (CPT2). Such an adaptation resulted in a greater utilization of fatty acids, increasing overall exercise capacity. Although these adaptive processes are important and signify the versatility of the cell to environmental stress, adequate mitochondrial biogenesis and remodeling is imperative for sustained health and with increased workload.

There is ample evidence within the field signifying how the upregulation of mitochondrial biogenesis following exercise training is strongly associated with increased mitochondrial fusion [73], in line with an increase in exercise performance and training capacity. The increase in mitochondrial fusion following exercise training is consistently reported in the literature, either by increased expression of Mfn1, Mfn2, and/or OPA1 or a decrease in the activity of regulators of mitochondrial fission [74–76]. This is supported by Fealy *et al.* [77] who found that 12 weeks of exercise training decreased the activation of pDrp1^{S616} in insulin-resistant human skeletal muscle, thereby promoting a mitochondrial fusion phenotype. Other studies have reported an increase in skeletal muscle OPA1 protein expression following acute or chronic exercise.

Although aerobic exercise is predominantly associated with mitochondrial fusion, there is evidence that aerobic exercise jointly, although to a lesser extent, increases mitochondrial fission

[75]. Such evidence supports the dynamic nature these proteins play within the cell and physical activity's role in the maintenance of mitochondrial quality control. These studies support the observations of a more fused, tubular mitochondrial network following aerobic exercise training [74], reflecting a greater increase in mitochondrial fusion protein content compared with fission protein content [73,78].

As with aerobic exercise, resistance exercise training (full-body, 2 days/week, 10 weeks) has been shown to increase the expression of both fusion (Mfn1, Mfn2, and OPA1) and fission (Drp1) protein content in older adults. These changes are in line with an increase in proteins relating to OXPHOS [79], suggesting that resistance training may increase mitochondrial membrane surface area, enabling an increase in mitochondrial respiration, aiding cellular adaptation to the exercise stimulus [80].

In conjunction with the increased expression of proteins involved in mitochondrial biogenesis, the change in protein expression of fusion/fission proteins, along with enhanced mitochondrial oxidative capacity [12], is imperative for increasing mitochondrial content following exercise [73]. With this in mind, together with its prospective benefit in disease cessation, OPA1-specific enhancement therapies may also act as an exercise mimetic for health and disease, given its effect of increasing mitochondrial size and function aiding to increase aerobic capacity.

Concluding remarks and future perspectives

Optimal mitochondrial function and balanced mitochondrial dynamics are crucial for cardiac and skeletal muscle function. Not surprisingly, there is growing interest into the role of mitochondrial dynamics in cardiac failure and metabolic disease. More specifically, OPA1 has been proposed to be the initial step in mitochondrial elongation, subsequently increasing OXPHOS, comparable with what is observed during aerobic exercise. A reduction in OPA1 expression has been shown to lead to mitochondrial fragmentation, linked to skeletal muscle atrophy and cardiac failure in animal models. Studies investigating OPA1 adaptation in humans are sparse, although considering the intriguing findings to date in cell and animal models, more studies focusing on OPA1 regulation in humans are required to fully extrapolate the extent of OPA1 importance and the development of novel therapies for OPA1-specific disease progression, particularly in skeletal and cardiac muscle physiology.

There are multiple questions surrounding OPA1 that remain unanswered (see [Outstanding questions](#)), such as whether the changes in OPA1 gene expression are instigated by the (patho)physiological state or whether these states are a direct response to the changes in OPA1? Are the mechanisms involving OPA1 and skeletal muscle atrophy analogous to those relating to cardiac dysfunction and heart failure or are there tissue-specific differences? Can the (dys)regulation of OPA1 directly impair substrate oxidation, leading to metabolic disease progression such as T2DM, and does OPA1 directly regulate the secretion of FGF21? Further tissue-specific mechanistic studies into these areas may help to clarify these questions. Regardless of these questions, the literature to date clearly signifies the crucial role OPA1 has in the regulation of cellular homeostasis in skeletal and cardiac muscle.

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Declaration of interests

No interests are declared.

Outstanding questions

Are changes in OPA1 gene expression instigated by the (patho)physiological state or are these states a direct response to the changes in OPA1?

Are the mechanisms involving OPA1 and skeletal muscle atrophy analogous to those relating to cardiac dysfunction and heart failure or are there tissue-specific differences?

Can the (dys)regulation of OPA1 directly impair substrate oxidation leading to metabolic disease progression such as T2DM or is this an indirect effect?

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