Mitochondria in focus: From structure and function to their role in human diseases. A review

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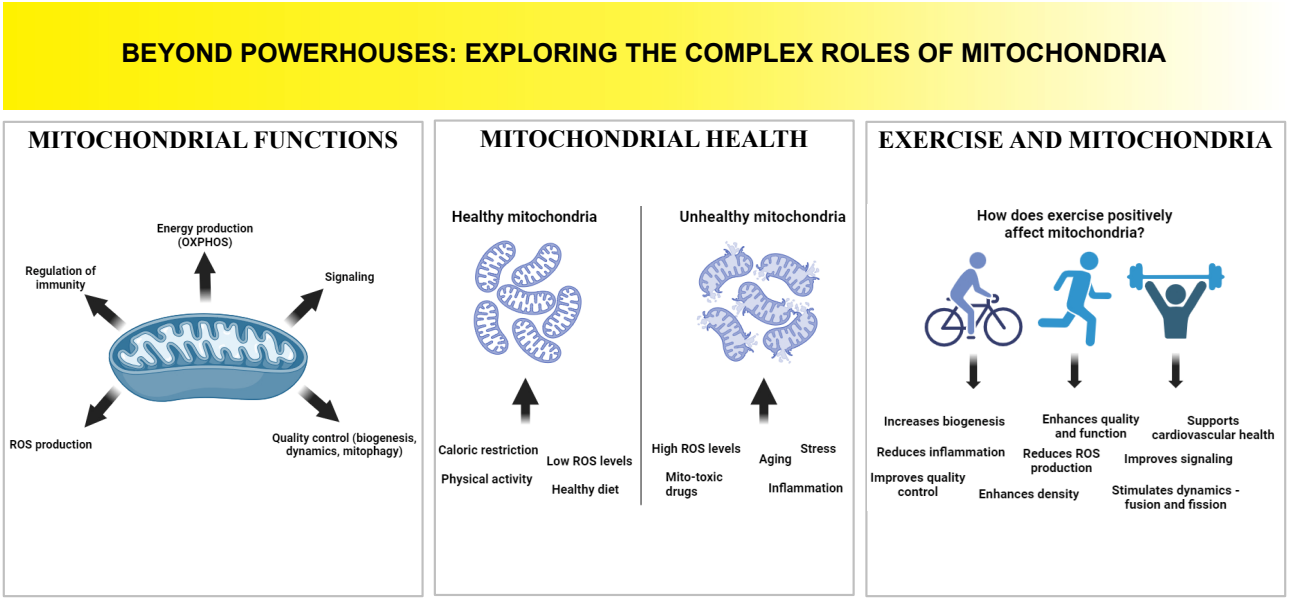
Mitochondria, double-membraned organelles within all eukaryotic cells, are essential for the proper functioning of the human organism. The frequently used phrase "powerhouses of the cell" fails to adequately capture their multifaceted roles. In addition to producing energy in the form of adenosine triphosphate through oxidative phosphorylation, mitochondria are also involved in apoptosis (programmed cell death), calcium regulation, and signaling through reactive oxygen species. Recent research suggests that they can communicate with one another and influence cellular processes. Impaired mitochondrial function on the one hand, can have widespread and profound effects on cellular and organismal health, contributing to various diseases and age-related conditions. Regular exercise on the other hand, promotes mitochondrial health by enhancing their volume, density, and functionality. Although research has made significant progress in the last few decades, mainly through the use of modern technologies, there is still a need to intensify research efforts in this field. Exploring new approaches to enhance mitochondrial health could potentially impact longevity. In this review, we focus on mitochondrial research and discoveries, examine the structure and diverse roles of mitochondria in the human body, explore their influence on energy metabolism and cellular signaling and emphasize their importance in maintaining overall health.



Mitochondria are vital organelles for energy metabolism, signaling, and cellular health, with their dysfunction linked to aging and diseases. This review highlights their roles and health potential.

Follprecht D. et al. doi: 10.5507/bp.2025.009

Graphical Abstract

Biomedical Papers

https://biomed.papers.upol.cz

Key words: mitochondria, oxidative phosphorylation, reactive oxygen species, mitochondrial function, mitochondrial disease

Received: December 3, 2024; Revised: March 10, 2025; Accepted: March 19, 2025; Available online: April 14, 2025
https://doi.org/10.5507/bp.2025.009

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INTRODUCTION

These days, we are witnessing constant progress in the field of biology and medicine, which helps us to better understand the complex mechanisms of the human body functioning. One of the key players in this vast system is the mitochondrion, a small ubiquitous organelle ~0.5 to 3 μm in size, variously shaped, and found within the cell cytoplasm[1]. Mitochondria are primarily responsible for cellular respiration by oxidizing carbohydrates and fatty acids to generate adenosine triphosphate (ATP) through oxidative phosphorylation (OXPHOS) (ref.[2]). However, they are far more than just energy production units. Their role in energy metabolism is well known, but we are still uncovering new aspects of their nature. Mitochondria have a key influence on signaling pathways in cells, affecting various biological processes, including apoptosis, stress response, and even the regulation of gene expression[3,4]. Their ability to communicate with other organelles or the nucleus and respond to various stimuli from the external environment makes them a fascinating center of cellular activity[5].

BRIEF HISTORY OF MITOCHONDRIAL RESEARCH

The historical significance of mitochondria research in cell biology spans several centuries and involves the contributions of numerous scientists. Given the nature of this review, only selected discoveries will be presented. In the 17th century, Robert Hooke and Antoni van Leeuwenhoek were the first to observe microorganisms under a microscope, but the technology at that time was not sufficiently developed to fully understand their features and behavior[6]. More than 200 years later, in 1890, thanks to advanced technology, the German pathologist Richard Altmann introduced the term „bioblasts“ for granular structures living inside cells, later identified as mitochondria[7]. In 1898, the term “mitochondria” was aptly coined by the German anatomist Carl Benda, based on the Greek terms “mitos” (meaning thread) and “chondros” (meaning granule), due to the thread-like structure of mitochondria[8]. Research by B.F. Kingsbury and O. Warburg in 1912–1913 proved that mitochondria are the site of energy metabolism in eukaryotic cells[9,10]. These processes were later described in more detail by H. A. Krebs, who formulated the citrate cycle in 1937 and was awarded the Nobel Prize for this work[11]. This knowledge was primarily due to the isolation of individual coenzymes involved in these processes[8]. Belgian-American biologist Albert Claude used the first differential coagulation techniques to isolate mitochondria and proved that succinoxidase and cytochrome oxidase are associated with mitochondria[12]. After the invention of the electron microscope in the 1930s, the discovery of the structure and function of mitochondria became more accessible. In the 1950s, the first high-resolution images of mitochondria were published by George Palade and Fritiof Sjostrand[13]. Mitochondria were found to be surrounded by two membranes, and mitochondrial DNA (mtDNA) was discovered in 1963 by M. and S. Nass[14]. In 1967, Lynn Margulis hypothesized that mitochondria originated from free-living bacteria that had been ingested by another cell, a concept that became known as the endosymbiotic theory[15]. In 1988, the first two discoveries of mutations in mtDNA were associated with human disease. The first discovery was made by Anita Harding, who identified large-scale mtDNA deletions in muscle biopsies of patients with “mitochondrial myopathies”. The second discovery, made by Doug Wallace, identified a point mutation in the mtDNA gene for NADH dehydrogenase complex subunit 4 (MTND4), associated with maternally inherited Leber hereditary optic neuropathy (LHON). These discoveries started extensive research in the field of mitochondrial diseases[16].

MITOCHONDRIAL STRUCTURE

Mitochondria are surrounded by a phospholipid double membrane, consisting of an outer mitochondrial membrane (OMM) and an inner mitochondrial membrane (IMM), characterized by different compositions, permeabilities and features. The area between the OMM and the IMM is called perimitochondrial or mitochondrial intermembrane space (IMS). The center of the mitochondrion, bounded by the IMM, is called mitochondrial matrix. These aqueous sub-compartments (IMS and mitochondrial matrix), with different pH values of 7.2–7.4 and 7.9–8.0, respectively, have a shallow proton gradient between them, drive ATP production via ATP synthase, and are involved in important processes responsible for proper mitochondria and cellular functioning[17,18].

The OMM separates the organelle from the cell cytoplasm. It is a porous protective barrier containing transport proteins called mitochondrial porins, which allow the passage of ions and small molecules through the membrane[17]. OMM also participates in the continuous process of mitochondrial fusion and fission, referred to as mitochondrial dynamics, which is an important element of changes in mitochondrial morphology[19,20]. Moreover, the outer mitochondrial membrane forms a reversible physical connection with the endoplasmic reticulum (ER), called mitochondria-associated membranes (MAM), which mediates communication between these two organelles[21]. The close juxtaposition between the ER and OMM regulates the functions of these organelles and ensures calcium (Ca2+) signaling, lipid biosynthesis, autophagy, and apoptosis. These connections fundamentally affect the life and death of the cell itself[22].

The mitochondrial IMS, only about 60 nm in diameter, ensures the exchange of proteins, lipids, metabolites, metals, and cofactors necessary for proper redox control. Some IMS proteins assist in respiratory chain assembly and maintain chain activity, while others trigger apoptosis[3,23]. While we lack information about some IMS proteins, the predominant import pathway is well described, like other mitochondrial compartments. However, novel specific (sub) pathways are still being characterized, and many aspects of mitochondrial physiology remain unex-

plored. Understanding the degradation and export of IMS proteins is also crucial for maintaining IMS balance[24].

The IMM, enclosing the matrix space, is a diffusion barrier requiring transport proteins to help small solutes like ions and metabolic substrates pass through. Moreover, with its protein-to-lipid mass ratio of ~75:25, it is one of the most protein-rich bilayers in biological systems. This membrane is involved in processes such as OXPHOS, protein translocation, assembly and degradation, iron-sulfur biogenesis, or metabolic exchange[25]. The IMM is wrinkled into characteristic folds termed cristae, which extend into the mitochondrial matrix and are shaped by various membrane-shaping proteins, including the mitochondrial contact site and cristae organizing system (MICOS) with many different subunits. This system also establishes contacts between the OMM and IMM, and it is a key multiprotein complex that regulates cristae biogenesis and the organization of the IMM into cristae. A convincing clue pointing to the involvement of MICOS in cristae morphogenesis is derived from evolutionary studies, which reveal that MICOS components are primarily present in organisms with mitochondria that have cristae structures[19].

Mitochondrial matrix, the space within the IMM, acts as a site for energy producing processes. It also contains mtDNA, ribosomes, soluble enzymes, nucleotide cofactors, small organic molecules and inorganic ions[26]. The tricarboxylic acid (TCA) cycle, also known as the Krebs cycle, takes place in the matrix and is a closed loop of reactions that releases energy stored in macronutrients through the oxidation of acetyl-CoA. The TCA cycle generates the reducing equivalents nicotinamide adenine dinucleotide (NADH), which enters the electron transport chain (ETC), a series of protein complexes and other molecules that transfer electrons from donors to higher redox potential acceptors via redox reactions[27]. This process simultaneously couples electron transfer with the transfer of H+ ions across the IMM until electrons are passed to oxygen (O2), the terminal electron acceptor in complex IV. As this process requires oxygen, it is called oxidative phosphorylation[28].

MITOCHONDRIAL QUALITY CONTROL

Mitochondrial quality control (MQC) involves processes such as mitochondrial biogenesis, proteostasis, dynamics, and/or mitophagy, all of which participate in cell homeostasis and proper mitochondrial functioning. In the case of failure of these processes, mitochondrial dysfunction can occur, which is considered to be the basic mechanism of aging, the development of cardiovascular diseases, neurological disorders, cancer, and/or prion disease[29].

Mitochondrial biogenesis

The process of mitochondrial biogenesis, which involves the generation of new mitochondria from those already existing, preserves the homeostasis of these organelles. In response to the energy demands triggered by developmental signals and environmental stressors, the cell initiates the process of mitochondrial biogenesis[30]. The master regulatory protein of mitochondrial biogenesis is the transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) (ref.[31]).

The nuclear genome encodes most of the mitochondrial proteins, which are translated in the cytosol and imported into mitochondria[32]. Protein homeostasis, or "proteostasis", within the mitochondria is a set of quality control machineries essential for the performance of mitochondrial functions. Any mutations or disturbances in proteostasis can cause human pathologies[33].

Mitochondrial dynamics – fusion and fission

Mitochondrial fusion is a cellular process whereby two or more mitochondria merge to form a single, larger mitochondrion. This multi-step process is essential for maintaining mitochondrial health and function within cells[34]. It involves many proteins in specific sequences, which occur on both mitochondrial membranes. While OMM fusion is mediated by the dynamin-like protein GTPases mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2), IMM fusion is promoted by dynamin-related optic atrophy protein 1 (OPA1) (ref.[19]). Mfn1 and Mfn2 share approximately 80% sequence similarity and possess similar functional domains, indicating that they contribute to metabolic functions by influencing mitochondrial structure. Their roles in this regard include both common and distinct functions[35]. Before fusion occurs, Mfn1 and Mfn2 form homotypic and heterotypic dimers to tether to the adjacent OMM, undergoing structural alterations prompted by nucleotide guanosine triphosphate (GTP) hydrolysis, which facilitates the fusion process[20]. A transmembrane protein, OPA1, performs two essential functions: preserving mitochondrial shape and energy levels by facilitating endosomal fusion and maintaining cristae structure. Deleting the OPA1 gene leads to mitochondrial fragmentation, whereas overexpressing OPA1 promotes elongation of mitochondria. For fusion, OPA1 is required solely in the IMM where two mitochondria are connected by trans-bolting. OPA1 gets incorporated into the inner membrane using its amino-terminal targeting sequence and transmembrane domain, enabling the blending of the mitochondrial matrix, DNA, and metabolites[36]. OPA1 exists in long and short isoforms. The long isoform is membrane-bound, while the short lacks a membrane anchor but can still interact with membranes. Under regular conditions, a combination of both isoforms is required for mitochondrial fusion; nevertheless, the long isoform alone can mediate fusion in cases of increased stress[37].

Mitochondrial fission is a process of dividing a single mitochondrion into two or more smaller mitochondria. Mitochondrial fission may serve to predispose depolarized or dysfunctional mitochondria to active turnover of fission products by mitophagy[38]. The main proteins that contribute to this process are dynamin-related protein 1 (DRP1), mitochondrial fission factor (MFF), mitochondrial fission protein 1 (FIS1), and mitochondrial dynamics proteins of 49 kDa (MiD49) and mitochondrial dynamics proteins of 51 kDa (MiD51) (ref.[19]). DRP1,

initially located in the cytosol, is recruited to the outer mitochondrial membrane (OMM) in response to specific stimuli, a process facilitated by receptors like MFF and FIS1. Once DRP1 connects to OMM, it creates multimers and rings. In this position, through the hydrolysis of GTP, the complex changes conformation and allows the constriction of the membrane to activate its division[4].

Mitophagy

As mitochondria play an essential role in many aspects of cell biology, several mechanistically distinct degradation pathways have evolved to maintain and modulate mitochondrial function. The selective form of autophagy called "mitophagy" responds to different signals, such as mitochondrial damage, excess mitochondria, or differentiation signals, to regulate the mitochondrial network's metabolic profile. This highly tuned process involves the capture of mitochondria in autophagosomes, which are transported to lysosomes for further degradation[39]. Various aspects of mitophagy signaling will be explored in this section.

Ubiquitin-mediated mitophagy

The proteins PTEN-induced kinase 1 (PINK1), Parkin, and ubiquitin exert an important role in this type of mitophagy. Under normal conditions, PINK1 levels are low in healthy mitochondria. However, when mitochondria become depolarized or damaged, PINK1 accumulates on their outer membranes. This leads to the phosphorylation of Parkin and ubiquitin, recruiting them to the impaired mitochondria. Autophagic receptor proteins such as p62 are then recruited into affected mitochondria, and finally, the removal of damaged mitochondria is mediated by lysosomes[36,40].

Receptor-mediated mitophagy

Fun14 domain protein 1 (FUNDC1), contained in mammalian cells, is a receptor for hypoxia-induced mitophagy located only on the OMM. FUNDC1 is phosphorylated to inhibit mitophagy under normal conditions[41]. However, mitophagy can be triggered by the dephosphorylation of FUNDC1 under anoxic conditions, which increases its interaction with the soluble microtubule-associated protein 1A/1B-light chain 3 (LC3) (ref.[42]). MicroRNA-137 (miR-137) can also regulate mitophagy through FUNDC1 (ref.[43]). FUNDC1-driven mitophagy might lead to boosted platelet activity, platelet cell death, and play a role in platelet clumping, adhesion molecule expression, or micro-thrombosis formation.

BNIP3 and BNIP3L (also known as NIX) are members of the BCL-2 family that induce mitophagy in three different ways. First, closely related to Parkinson's disease occurrence, NIX promotes the transport of Parkin to mitochondria, which can contribute to NIX ubiquitination[44]. Second, NIX recruits autophagic proteins (Atg8) to the damaged mitochondria, which induce mitophagy[45]. Third, as a member of the BCL-2 family, NIX triggers mitophagy by binding to proteins involved in autophagic vesicles. BNIP3 and NIX primarily trigger autophagy under hypoxic conditions. In this case, these proteins may initiate compensatory processes, allowing cell recovery from stress. Nonetheless, prolonged stress can lead to excessive autophagy and cell death[46].

Instead of degrading whole mitochondria, mitophagy can target the degradation of specific proteins or individual protein complexes in a process known as piecemeal mitophagy[47]. In piecemeal mitophagy, mitochondria bud off small vesicles that are engulfed by autophagosomes and are later transported to lysosomes for degradation. This process can be a very energy-efficient method to eliminate damaged mitochondrial components rather than whole mitochondria and represents a form of routine mitochondrial quality control[48,49].

Lipids like cardiolipin or ceramides can also act as receptors, initiating mitophagy. Cardiolipin is primarily localized in the IMM and, under specific mitochondrial stress conditions, it relocates to the OMM, where it can act as a receptor for the autophagy protein LC3B (ref.[50]). Ceramides are a family of bioactive lipids functioning either as signaling molecules or as structural components of membranes. The synthesis and mitochondrial localization of ceramides have been linked with the activation of lethal mitophagy, which can trigger cell death in cancer cell lines, such as acute myeloid leukemia (AML), or head and neck squamous carcinoma (HNSCC), and also in aging-stress-induced mitophagy in T cells[51-53].

ENERGY PRODUCTION IN MITOCHONDRIA

OXPHOS is the process of energy production in the form of adenosine triphosphate, which consists of a nitrogenous base (adenine), a ribose sugar, and three serially bonded phosphate groups. This process generates approximately 32 ATP molecules per one molecule of glucose oxidized. It occurs primarily in the IMM and fuels essential cellular functions, including muscle contractions or brain activities[54,55]. Aside being the primary source of energy for cells, ATP is the paramount molecule driving virtually all cellular activities and also plays a role in several biological processes, including DNA and RNA synthesis, ion-channel regulation, intercellular signaling mediation and protein phosphorylation[56]. At the heart of mitochondria within the mitochondrial cristae, the ETC uses oxidation-reduction reactions to move electrons between protein complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex III (cytochrome c reductase) and complex IV (cytochrome c oxidase). Protein complex V, also known as F_1F_0-ATP synthase, which is the main contributor to cristae structure, catalyzes the formation of ATP by using ADP and inorganic phosphate (P_i) in a process called chemiosmosis[17,57,58]. The proton gradient in the ETC is generated mainly by protein complexes I, III, and IV. Complex I brings electrons from the soluble carrier molecule NADH into the respiratory chain and transfers them to quinol localized in the IMM. This electron transfer reaction releases energy used to pump four protons from the mitochondrial matrix into the cristae lumen. Complex III accepts electrons from the reduced quinol, subsequently transmitting them to cytochrome c,

Table 1. Key molecules involved in mitochondrial quality control (ref.[4,19,20,31,35,37,40–46,50–53,108]).

Molecule	Function
Atg8	Recruits damaged mitochondria to induce mitophagy
BNIP3	Induces mitophagy by promoting Parkin transport, recruits autophagic proteins to damaged mitochondria, triggers autophagy under hypoxic conditions
Cardiolipin	Acts as a receptor for the autophagy protein LC3B under specific mitochondrial stress conditions
Ceramides	Activates lethal mitophagy under stress conditions
DRP1	Facilitates mitochondrial fission
FIS1	Facilitates the recruitment of DRP1 to OMM
FUNDC1	Serves as receptor for hypoxia-induced mitophagy, interacts with LC3 under anoxic conditions, and is regulated by microRNA-137
LC3	Interacts with FUNDC1 under anoxic conditions to induce mitophagy
MFF	Recruits DRP1 to the OMM
MiD49, MiD51	Contribute to mitochondrial fission
Mfn1, Mfn2	Mediate OMM fusion
NIX	Induces mitophagy by promoting Parkin transport, recruits autophagic proteins to damaged mitochondria, triggers autophagy under hypoxic conditions
OPA1	Facilitates IMM fusion, maintains mitochondrial shape and energy levels
Parkin	Recruited to impaired mitochondria by PINK1 and ubiquitin, facilitates removal of damaged mitochondria
PGC-1α	Master regulatory protein of mitochondrial biogenesis
PINK1	Accumulates on depolarized or damaged mitochondria, phosphorylates Parkin and ubiquitin

the soluble electron carrier protein, and translocates four protons in the process via a redox loop. Complex IV facilitates the transfer of electrons from cytochrome c to molecular O2, simultaneously contributing to the proton gradient by translocating four protons (two pumped and two consumed) for every O2 molecule used to produce water. Complex II carries electrons from succinate to the quinol pool directly and does not contribute to proton translocation. Cytochrome c, which shuttles electrons from complex III to complex IV, can trigger apoptosis if released into the cytoplasm. Hence, it is necessary for cytochrome c to remain confined within the cristae, and for the OM to remain tightly sealed during mitochondrial dynamics[17,58]. Moreover, complexes I and III are considered the predominant sources of reactive oxygen species (ROS) in the respiratory chain. However, under certain circumstances, complex II, as well as other dehydrogenases utilizing the CoQ pool, can also contribute to the native rates of ROS production[59]. If ROS such as superoxide ($O2^{\bullet -}$) and hydrogen peroxide (H_2O_2) are overproduced in large enough quantities by mitochondria in mammalian cells, they are associated with pathogenesis of human diseases, including heart disease[60]. For example, increased production of $O2^{\bullet -}$ during reverse electron transfer (RET) from complex II to complex I has been associated with myocardial ischemia-reperfusion injury[61]. However, if produced at low, non-toxic levels for the cell, ROS can exert beneficial effects, leading to the improvement of mitochondrial function and, in this case, also act as physiological signaling molecules supporting the regulation of endogenous antioxidant enzymes and non-enzymatic ROS-scavenging systems (e.g., glutathione reductase, catalase, and peroxiredoxins, Mn-superoxide dismutase 2 (SOD2), and/or Cu/Zn-SOD). This concept of beneficial effects is called "Mitohormesis" (ref.[62]). Mitohormesis can be defined as a biological response to low doses of mitochondrial stress stimuli such as exercise, caloric restriction, intermittent fasting, and dietary phytonutrients, which can enhance lifespan, healthspan, and particularly improve metabolism and the immune system[63]. Reactive nitrogen species (RNS), which have similar features to ROS, are also considered side products of mitochondrial metabolism. Nitric oxide (NO) regulates oxygen consumption and ATP production in mitochondria, and its reaction with superoxide generates peroxynitrite (PeN), a potent oxidizing and nitrating agent. PeN, stabilized by the physiological alkaline mitochondrial environment, can diffuse into hydrophobic regions of mitochondrial membranes. It can cause oxidative damage through direct oxidation, nitration, or nitrosation of target molecules[64].

MITOCHONDRIAL SIGNALING

A crucial aspect of cellular function and homeostasis is the communication between mitochondria and the rest of the cell. As described by Picard & Shirihai (2022), mitochondrial signal transduction occurs in three key steps:

1. Sensing – Mitochondria detect metabolic and hormonal inputs, translating them into functional, biochemical, and morphological changes.
2. Integration – These inputs are processed through interactions between mitochondria and other organelles, influenced by the state of the mitochondrial network and the cell.
3. Signaling – Mitochondria generate outputs that regulate metabolic pathways, impact other organelles, and even influence nuclear gene expression. These signals can also act systemically, affecting physiological processes and organismal behavior.

Mitochondrial signal transduction also facilitates the interaction of organelle networks to perform complex cellular functions beyond the capabilities of isolated organelles[65]. Recent research over the past few decades has revealed mitochondria as multifaceted signaling organelles that ultimately determine whether cells survive. Mitochondrial signaling, whether under physiological or pathological conditions, can be characterized by Ca2+ signaling, oxidative stress, and ROS signaling in mitohormesis, anterograde (nucleus-to-mitochondria) and retrograde (mitochondria-to-nucleus) signal transduction, the role of mtDNA in immunity and inflammation, the triggering of mitophagy and apoptosis signaling pathways, and mitochondrial dysfunctions (mitochondriopathies) in cardiovascular, neurodegenerative, and malignant diseases[62]. The exchange of various signals is a highly complicated process, and many of these signals are tightly interlinked with each other. The variety of stimuli ranges from lipids, proteins, metabolites, gases, ions, and DNA to temperature and the interaction of mitochondria with other organelles. Stress stimuli are first sensed by mitochondria through ligand-activated receptors, biochemical reactions (OXPHOS), transporters, or the mitochondrial genome, and then these stimuli are converted into biochemical, functional, and/or morphological changes[66].

MITOCHONDRIA AND DISEASES

Mitochondrial diseases (MD) comprise a diverse group of acquired and/or genetic disorders characterized by mitochondrial dysfunction. These disorders, also called mitochondriopathies, arise from mutations in mtDNA or nuclear DNA (nDNA) and are often associated by high morbidity and mortality. Mitochondriopathies primarily affect high-energy-demand tissues, such as the brain, retina, kidney, liver, and muscles, leading to symptoms including neurodegeneration, muscle weakness, cardiomyopathy, optic atrophy, and liver failure[67]. From neurological disorders to metabolic abnormalities, MD present a complex challenge for diagnosis and treatment. Understanding the complex interplay of genetic and envi-

Table 2. Mitochondrial diseases (adapted and modified from ref.[109,110]).

Syndrome	Age at onset	Clinical features	Predominant mutations
Chronic progressive external ophthalmoplegia (CPEO)	Early adult	External ophthalmoplegia and bilateral ptosis. Other features: proximal myopathy, cardiac conduction defects and dysphagia, etc.	Large-scale single mtDNA deletions
Kearns–Sayre syndrome (KSS)	Later childhood	Progressive external ophthalmoplegia, pigmentary retinopathy, cerebellar ataxia, raised cerebrospinal fluid protein, cognitive impairment, deafness, cardiomyopathy, dysphagia, etc.	Large-scale single mtDNA deletions
Leber hereditary optic neuropathy (LHON)	Adult	Optic neuropathy with sub-acute bilateral deterioration in vision.	m.3460G>A (ND1), m.11778G>A (ND4), m.14484T>C (ND6)
Maternally inherited Leigh syndrome (MIILS)	Infancy and early childhood	Developmental delay, muscle weakness, problems with movement, seizures, myoclonus, heart disease, kidney problems, difficulty in breathing, etc.	m.8993T>G, m.8993T>C (MT-ATP6)
Mitochondrial encephalopathy with lactic acidosis and stoke-like episodes (MELAS)	Late childhood or adulthood	Stroke-like episode with seizures. Other features: intermittent episodes of encephalopathy, vomiting, migraine, diabetes mellitus, cardiomyopathy, pigmentary retinopathy, etc.	m.3243A>G (MT-TL1), m.3271T>C (MT-TL1), m.1642G>A (MT-TV), m.9957T>C (MT-CO3), m.1277A>G, m.13045A>C, m.13513G>A, m.13514A>G (MT-ND5)
Myoclonic epilepsy with ragged red fibers (MERRF)	Childhood or early adulthood	Cerebellar ataxia and myopathy, myoclonus epilepsy. Other features: dementia, optic atrophy, bilateral SNHI, pyramidal signs, spasticity, etc.	m.8344A>G, m.8356T>C, m.8363G>A (MT-TK), m.3291T>C (MT-TL1), m.4279A>G (MT-TI), mutations in MT-TF (tRNA Phe) and MT-TP (tRNA Pro)
Neuropathy, ataxia and retinitis pigmentosa (NARP)	Late child or adult	Peripheral neuropathy, pigmentary retinopathy, ataxia.	m.8993T>G, m.8993T>C (MT-ATP6)
Pearson syndrome	Early childhood	Sideroblastic anemia, pancytopenia, exocrine pancreatic failure, renal tubular defects, etc.	Large-scale single mtDNA deletions

ronmental factors in mitochondrial dysfunction is essential for detecting these disorders and developing targeted therapeutic interventions[68]. MD in childhood represent complex clinical, biochemical, and genetic problems that include both classical syndromes and non-classical multisystem manifestations. The complexity of the diagnosis results from the unique clinical features of each child. Although individually rare, MDs in adults are collectively among the most common genetic diseases, exhibiting genotype and phenotype complexity across the nuclear and mitochondrial genomes[69,70].

Primary mitochondrial diseases

Primary mitochondrial diseases (PMD) can result from germline mutations in mtDNA and/or nDNA genes that encode ETC proteins. Point mutations in any of the 37 mtDNA genes, which encode 13 proteins, 22 transfer RNAs (tRNA), and 2 ribosomal RNAs (rRNA), can affect the proper functioning of the ETC (ref.[71]). Approximately 250–300 genes are thought to regulate the ETC from the nucleus, while about 1,500 nuclear genes contribute to broader mitochondrial processes. These functions encompass activities beyond the ETC, including fatty acid oxidation and the Krebs cycle[72]. PMD arise not only from defective ETC protein-encoding genes but also from germline mutations in other nDNA genes. These mutations impact OXPHOS function by disrupting the production of complex machinery essential for optimal ETC performance[73]. Over the past 30 years, mitochondrial medicine has identified nearly 400 genes linked to PMD. Despite this progress, challenges remain in understanding tissue specificity, clinical variability, and the lack of disease-modifying therapies. Research has expanded our understanding of mitochondrial function, highlighting its relevance across medical specialties[69,70]. Examples of major PMD clinical phenotypes primarily arising from mutations in mtDNA include:

Leber's hereditary optic neuropathy (LHON); Mitochondrial encephalopathy, lactic acidosis, stroke-like syndrome (MELAS); Kearns–Sayre syndrome (KSS); Myoclonus, epilepsy, ragged-red-fibers syndrome (MERRF); Neuropathy, ataxia, retinitis pigmentosa (NARP)/maternally inherited Leigh syndrome (MIILS); Maternally inherited non-syndromic deafness (associated or not with aminoglycoside use); Chronic progressive external ophthalmoplegia (CPEO) spectrum; Pearson's syndrome[69]. List of individual predominant mutations in table 2.

Secondary mitochondrial dysfunction

In contrast, secondary mitochondrial dysfunctions (SMD) are caused by mutations in genes unrelated to OXPHOS. SMD can also be acquired through various conditions, including disruptions in pathways regulating mitochondrial functions or exposure to factors (e.g., mitotoxic drugs, inflammatory responses, aging) that cause oxidative stress and can affect or worsen neurodevelopmental and neurodegenerative diseases, such as diabetes, Parkinson's disease, Alzheimer's disease, heart and kidney diseases, and/or cancer. Unlike PMD, SMD can be either inherited or acquired. However, distinguishing between inherited and acquired mitochondrial dysfunction is complex, as some primary mitochondrial diseases also arise from spontaneous de novo mutations, which are technically acquired[73,74].

Mitochondria and cancer

Mitochondria play a crucial role in tumorigenesis, as functional OXPHOS is essential for tumor growth[75]. Despite the Warburg effect, tumors rely on mitochondrial bioenergetics, and disruption of the ETC shows therapeutic promise[76]. Cells lacking mtDNA fail to form tumors unless they acquire host mtDNA, restoring respiration[77]. Interestingly, ATP production is not essential for tumorigenesis; instead, OXPHOS-driven de novo pyrimidine biosynthesis via dihydroorotate dehydrogenase (DHODH) is critical[78]. This highlights the importance of mitochondrial mechanisms, such as the phenomenon of horizontal mitochondrial transfer (HMT), which has been implicated in cancer progression. HMT supports tumor onset and rapid growth by facilitating processes like OXPHOS-driven pyrimidine biosynthesis. While the clinical relevance of HMT is currently limited, ongoing research suggests that targeting this process could provide new strategies for disrupting tumor growth and overcoming therapy resistance[79]. Targeting DHODH or ETC Complex III may provide new therapeutic strategies by blocking pyrimidine synthesis and increasing oxidative stress, offering a dual approach to impair tumor growth[80].

The diagnostic value of the mitochondrial genome in mitochondrial diseases (MD) is crucial, especially when the nuclear origin remains unclear. While muscle biopsy has traditionally been considered the gold standard for diagnosing MD in both children and adults, its invasive nature has led to a shift in its role in modern diagnostic practices. Although muscle biopsy still provides the most comprehensive morphological, biochemical, and molecular insights – identifying mtDNA deletions or point mutations – it is increasingly being supplemented or replaced by less invasive alternatives. For many mtDNA variants with established pathologies, molecular analysis of blood or hair follicles, which can reveal important information about heteroplasmy, is now considered sufficient[81,82]. These less invasive methods, including urinary epithelium and skin fibroblasts, are particularly useful in experimental studies and are gaining recognition in clinical diagnostics. Tissue selection remains critical for identifying pathogenic mtDNA variants, following the specific principles of mitochondrial medicine[82,83].

Genetic disorders often result from mitochondrial dysfunction, but diagnosing suspected cases remains a significant challenge. Despite progress, understanding genotype-phenotype connections and developing effective therapies are still ongoing. Future research should focus on unraveling mitochondrial biology to identify the molecular and cellular bases of mitochondrial diseases and potential targets for new treatments[84].

EFFECTS OF AGING ON MITOCHONDRIA

Every living organism undergoes a complex biological process of aging with detrimental effects on its physiology, notably on mitochondrial metabolism. This decline in mitochondrial functions, characterized by an elevated mtDNA mutation rate, heightened ROS production, reduced efficacy of the ETC, defective cytoplasmic Ca2+ regulation, and unregulated release of pro-apoptotic factors, is a hallmark of aging, emphasizing the pivotal role of mitochondria in maintaining organismal health[85]. ROS produced by mitochondria are thought to be a key factor contributing to mitochondrial damage during aging[86]. Aging and age-related diseases share a common factor: inflammation, which contributes to the pathogenesis of these conditions. This long-term, chronic, sterile inflammation (taking place without infection and predominantly driven by endogenous signals) is referred to as "inflammaging". In addition to persistent bacterial and viral infections, misfolded and oxidized proteins, cellular debris, and misplaced molecules are major contributors to inflammaging[87]. The development of this condition, marked by elevated inflammatory biomarkers such as IL-6 and C-reactive protein in the elderly, is a known risk factor for increased morbidity and mortality[88]. Moreover, aging, as well as severely reduced physical activity levels (muscle disuse), leads to muscle atrophy known as "sarcopenia", which is linked to decreased mobility, increased risk of falls, and the onset of obesity and diabetes[89,90]. Another factor contributing to mitochondrial dysfunction and sarcopenia is the accumulation of age-related mtDNA mutations. Evidence indicates that muscle fibers with over 80% of their mtDNA harboring mutations have a higher likelihood of muscle fiber breakage and atrophy[91]. Aging is also accompanied by increased levels of lysosomal proteins, including Lamp1, Lamp2, and cathepsin D. These elevations may be necessary to assist with the breakdown of damaged cellular components during periods of muscle disuse[89]. Targeting mitochondrial health has emerged as a potential strategy for mitigating age-related decline and promoting healthy aging, which can be achieved in several ways. For example, caloric restriction preserves mitochondrial function in aging skeletal muscles and maintains the integrity of DNA and the proteome. In contrast, caloric restriction does not increase mitochondrial biogenesis[92]. Endurance exercise, the most powerful physiological stimulant of mitochondrial biogenesis in skeletal muscle, also exerts profound effects on metabolism in other tissues,

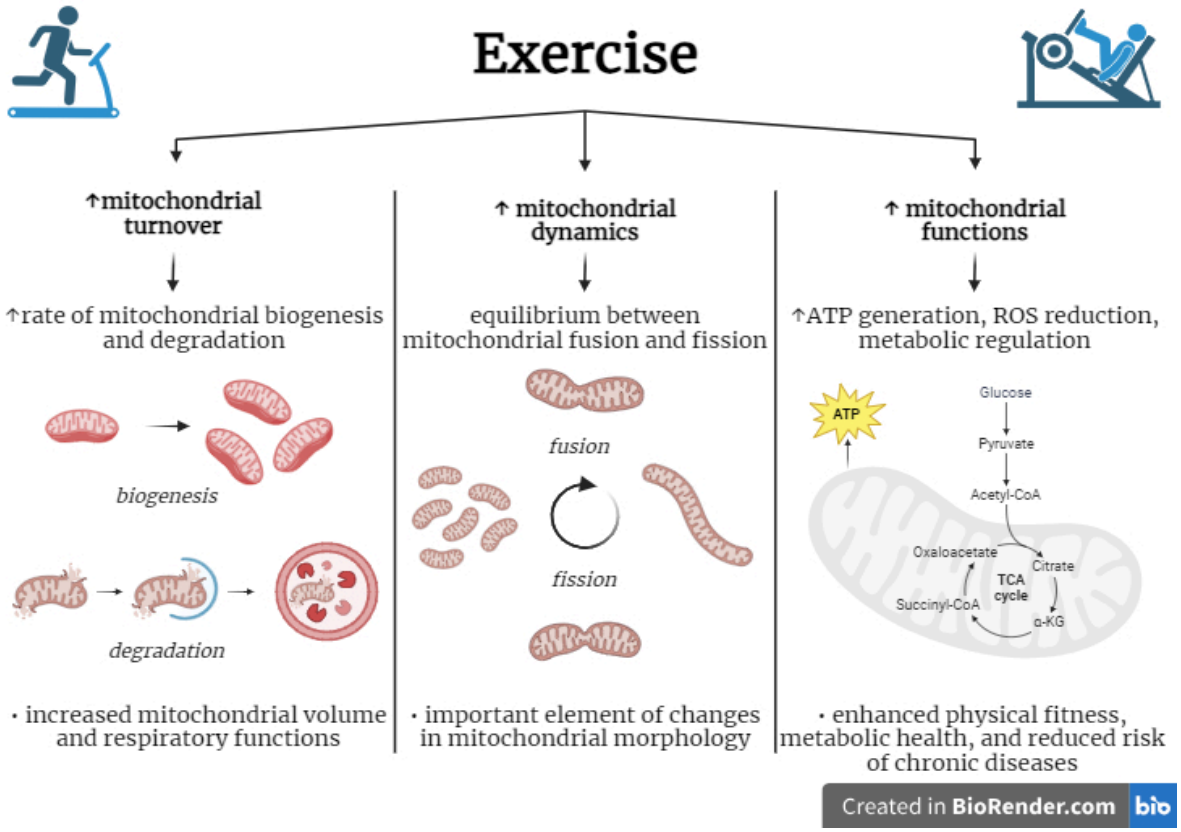

Fig. 1. Schematic overview of the effects of exercise on mitochondria – Exercise influences three key aspects of mitochondrial health in skeletal muscle: turnover, dynamics, and functions. Exercise increases mitochondrial turnover by enhancing the rate of biogenesis and degradation, leading to greater mitochondrial volume and improved respiratory function. It also maintains the equilibrium between mitochondrial fusion and fission, which is vital for changes in mitochondrial morphology. Additionally, exercise boosts mitochondrial functions, resulting in increased ATP generation, reduced reactive oxygen species (ROS) production, and improved metabolic regulation, thereby enhancing physical fitness, metabolic health, and reducing the risk of chronic diseases. Image created with BioRender.com.

including the brain, heart, liver, and adipose tissue. These adaptations lead to improved healthspan, reduced risk of morbidity and mortality, and extended life expectancy[93].

EXERCISE AND MITOCHONDRIA

Endurance exercise serves as a powerful stimulus impacting both cytoplasmic and nuclear proteins as well as genes encoding mitochondrial proteins. Depending on the nature of the exercise, these effects lead to higher mitochondrial biogenesis, which is associated with increased mitochondrial volume and respiratory function[94]. Exercise increases skeletal muscle energy demand and initiates more efficient processes for regulating mitochondrial turnover, specifically macroautophagy and lysosomal activity, while maintaining equilibrium between mitochondrial biogenesis, dynamics, and mitophagy. During high-intensity exercise, the increased metabolic demands accelerate glycolysis, which raises lactate and pyruvate concentrations. This leads to a significant increase in the lactate/pyruvate (L/P) ratio. As a result, more lactate is transported into the mitochondrial reticulum, influencing cellular metabolism[95,96]. Recent research suggests that exercise not only enhances mitochondrial function but also stimulates mitochondrial turnover, improving both their quality and efficiency. Study from Bishop et al.[97] suggests that exercise intensity predominantly influences improvements in mitochondrial function, measured by mitochondrial respiration, whereas exercise volume is a key determinant of exercise-induced improvements in mitochondrial mass. Additional research, involving direct comparisons of various training intensities and volumes within the same study is necessary to verify these observations. During exercise, muscle contractions trigger various hormetic pathways, including improved nutrient-sensor AMP kinase activity, modulation of transcriptional PGC-1α, mTOR regulation, sirtuin 1 signaling, and activation of intracellular PI3K-Akt. These mechanisms collectively coordinate adaptations in response to exercise[5]. Performing different types of exercise appears to be a highly effective strategy in combating a plethora of diseases. Physical activity, and conversely, physical inactivity are major environmental modulators of cardiorespiratory fitness (CRF). Endurance exercise is widely acknowledged for its positive impact on CRF and cardiometabolic risk factors. Exercise improves multiple factors, such as VO2max, oxygen transport capacity, O_2 diffusion to working muscles, and ATP generation (e.g., mitochondrial density) (ref.[98]).

Exercise plays a crucial role in enhancing the function and efficiency of different types of mitochondria in skeletal muscle, specifically subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria. These adaptations are essential for improving muscle endurance, performance, and overall metabolic health, as exercise-induced mitochondrial biogenesis and increased oxidative capacity contribute to more efficient ATP production and utilization within muscle fibers[99,100]. Subsarcolemmal mitochondria are a more reticular type of organelle positioned near the capillaries and nuclei beneath the sarcolemmal membrane, with interconnections between neighboring mitochondria that form an irregular network of organelles. SS mitochondria contribute to ATP provision for membrane active transport and also supply energy for nuclear processes such as transcription and transport of nuclear molecules. Meanwhile, intermyofibrillar mitochondria, located between myofibrils adjacent to the Z-line, are instrumental in supplying ATP to contractile filaments for muscle contraction and participating in Ca2+ signaling, due to their proximity to the transverse tubules and sarcoplasmic reticulum release units. SS mitochondria display a punctate appearance, while IMF mitochondria present elongated structures, and both morphologies are actively modulated in response to metabolic requirements[89,101–103]. Understanding the specific impacts of exercise on SS and IMF mitochondria highlights the importance of regular physical activity in maintaining and enhancing muscle function.

Physical activity also facilitates multi-tissue crosstalk mediated by substances called exerkines, such as interleukin-6, irisin, apelin, fibroblast growth factor, or adiponectin[104]. Recent studies have shown that exerkines secreted in response to exercise from various organs and tissues mediate multisystemic health benefits. Evidence indicates extensive involvement of exerkines in the regulation of mitochondrial functions. Besides exerkines, several stress hormones, including glucocorticoids and catecholamines, are released during exercise, and mitochondria participate in both the synthesis and metabolism of these hormones. In summary, exercise induces significant mitochondrial adaptations, enhancing both quality and quantity through mechanical stress, ROS, nitric oxide (NO), and substrate availability in the vasculature. These modifications improve mitochondrial bioenergetics, cellular redox balance, mitochondrial signaling, while decreasing cellular vulnerability to harmful stimuli[105]. Conversely, engaging in excessive exercise beyond a certain threshold leads to mitochondrial dysfunction and glucose intolerance, as evidenced by a study showing a partial shutdown of mitochondrial respiration and H_2O_2 production during intensive training[106]. Mitochondria respond to exercise and caloric restriction (CR) by enhancing cellular energy production and metabolic efficiency. The combined effects of exercise and CR have been shown to alter mitochondrial biogenesis and function, leading to enhanced cellular resilience and overall metabolic health[107].

CONCLUSION

In conclusion, the roles of mitochondria extend far beyond energy production, encompassing vital functions in cell dynamics, signaling, and maintaining organismal integrity. Their structural complexity and dynamic behavior underscore their indispensability in cellular processes. Moreover, their involvement in aging, response to exercise, and susceptibility to human mitochondrial diseases highlight their importance in health and disease. Many

questions about mitochondria remain unanswered, making further research essential to fully comprehend their complexities. Expanding our knowledge of mitochondria promises not only to deepen our understanding of fundamental cellular processes but also to drive innovative therapeutic interventions targeting mitochondrial dysfunction in various diseases.

Acknowledgement: This work was supported by the Cooperatio Program, research area IMMU.

Author contributions: DF: manuscript drafting, literature review, content writing, interpretation of findings; JV: literature search, study selection, text contribution on exercise and mitochondria; VJ: critical review, clarity improvement, feedback on mitochondrial mechanisms and metabolism sections; PB: theoretical input, methodological suggestions, table creation, manuscript finalization; AK: supervisor, critical article review, methodological corrections, content finalization.

Conflict of interest statement: The authors state that there are no conflicts of interest regarding the publication of this article.

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