# Mitochondria and Sex-Specific Cardiac Function

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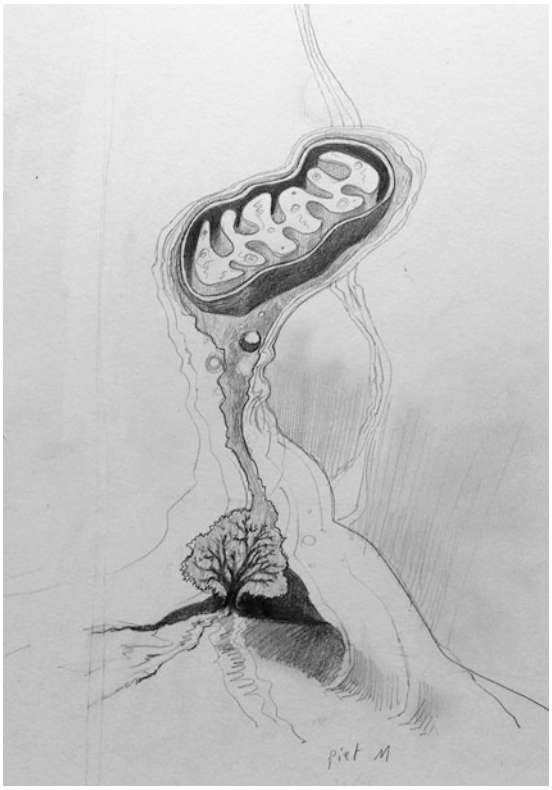
Rosa Vona, Barbara Ascione, Walter Malorni, and Elisabetta Straface




*Cardiac mitochondria. Art work by Piet Michiels, Leuven, Belgium*

**Abstract**

The focus of this chapter is the gender differences in mitochondria in cardiovascular disease. There is broad evidence suggesting that some of the gender differences in

R. Vona · B. Ascione · W. Malorni · E. Straface (✉)
Biomarkers Unit, Center for Gender-Specific Medicine, Istituto Superiore di Sanità, Rome, Italy
e-mail: rosa.vona@iss.it; barbara.ascione@iss.it; malorni@iss.it; elisabetta.straface@iss.it

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cardiovascular outcome may be partially related to differences in mitochondrial biology (Ventura–Clapier R, Moulin M, Piquereau J, Lemaire C, Mericskay M, Veksler V, Garnier A, Clin Sci (Lond) 131(9):803–822, 2017)). Mitochondrial disorders are causally affected by mutations in either nuclear or mitochondrial genes involved in the synthesis of respiratory chain subunits or in their posttranslational control. This can be due to mutations of the mtDNA which are transmitted by the mother or mutations in the nuclear DNA. Because natural selection on mitochondria operates only in females, mutations may have had more deleterious effects in males than in females (Ventura–Clapier R, Moulin M, Piquereau J, Lemaire C, Mericskay M, Veksler V, Garnier A, Clin Sci (Lond) 131(9):803–822, 2017; Camara AK, Lesnefsky EJ, Stowe DF. Antioxid Redox Signal 13(3):279–347, 2010). As mitochondrial mutations can affect all tissues, they are responsible for a large panel of pathologies including neuromuscular disorders, encephalopathies, metabolic disorders, cardiomyopathies, neuropathies, renal dysfunction, etc. Many of these pathologies present sex/gender specificity. Thus, alleviating or preventing mitochondrial dysfunction will contribute to mitigating the severity or progression of the development of diseases. Here, we present evidence for the involvement of mitochondria in the sex specificity of cardiovascular disorders.

**Keywords**

Mitochondrial biology · Mutations of mtDNA · Mitochondrial dysfunction · Krebs cycle · Nuclear respiratory factor · Apoptotic bodies · Redox messenger · Reactive oxygen species · Calcium overload · Heart failure · Ischemic preconditioning · Autophagy · Mitophagy · Dynamin · Aging heart

## Mitochondrial Functions

Mitochondria are crucial, multifunctional organelles, which actively regulate cellular homeostasis. They have a double membrane. The composition of the outer membrane is similar to that of other eukaryotic membranes. The inner membrane resembles prokaryotic membranes in composition and physical properties [102]. In the matrix, mitochondria contain a circular small genome (mtDNA) encoding for 13 proteins of the respiratory chain and for both ribosomal ribonucleic acid (rRNA) and transfer RNA (tRNA). Mutations in mtDNA result in severe neuromuscular diseases, mostly due to impaired energy production. The main function of mitochondria is the energy production as adenosine triphosphate (ATP) via citric cycle (tricarboxylic acid cycle, Krebs cycle). Other cell functions include ionic homeostasis, production and regulation of reactive oxygen species (ROS), pH regulation, steroid hormone synthesis, calcium homeostasis, thermogenesis, lipid and carbohydrate utilization, and cell death [82, 98]. Mitochondria proliferate by division of preexisting organelles, a process called mitochondrial biogenesis. This *process* is under the control of the nucleus and necessitates coordination of the nuclear and mitochondrial ones genomes. The transcription and replication of the mitochondrial genome are activated by the nuclear-encoded mitochondrial transcription factor A (TFAM). In turn, TFAM transcription is activated by the nuclear respiratory factors (NRF) 1 and 2 and the peroxisome proliferator-activated receptor γ coactivators 1 (PGC-1α or β), the master regulators of mitochondrial biogenesis. These coactivators and transcription factors also coordinate the expression of multiple nuclear-encoded mitochondrial proteins, which have to be processed, imported, and localized in the proper mitochondrial compartment with mitochondria-encoded proteins [122].

Mitochondria are essential for cardiac function. They play a major role in ATP supply, needed to support continuous cycles of contraction and relaxation, carry out synthesis of essential cellular components, calcium buffering [28], and trigger cell death signals [27]. Because the heart is highly dependent on mitochondrial oxidative energy, it is understandable that defects in mitochondrial structure and function can be found in association with different cardiovascular diseases. Indeed, abnormalities in the

mitochondrial function cause cardiomyopathies, arrhythmias, and abnormalities of the conduction system. Mitochondrial dysfunction has been linked to several cardiovascular disorders, including hypertension, cardiac hypertrophy, ischemia/reperfusion, and heart failure [61, 117]. Importantly, mitochondria are directly involved in triggering of different and complexly interconnected programs controlling cell "fate" such as apoptosis, autophagy (mitophagy), and senescence, all involved in cardiovascular disorders [2]. Below will be expanded the individual programs in detail and their implications in cardiovascular diseases.

## Mitochondria and Apoptosis

Mitochondria are instrumental for cell life and death. They play a central role in various forms of cell death, which are characterized by differential biochemical features, with predominant forms including apoptosis (caspase-dependent and caspase-independent), or necrosis. Besides amplifying and mediating extrinsic apoptotic pathways, mitochondria also play a central role in the integration and propagation of death signals originating from inside the cell such as DNA damage, oxidative stress, starvation, as well as those induced by radiation or chemotherapy [68]. Apoptosis, also known as programmed cell death, describes a particular mode of cell death that is characterized by a series of biochemical events that lead to a variety of morphological changes, including cell shrinkage, membrane blebbing or budding, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. Ultimately, the cell is fragmented into compact membrane-enclosed structures, called "apoptotic bodies," which contain cytosol, condensed chromatin, and organelles. Apoptotic bodies are recognized by phagocytes and quickly removed preventing inflammation and tissue damage that might ensue upon cell lysis [1, 39]. Apoptosis is induced via two main ways involving either the mitochondria (the intrinsic pathway) or the activation of death receptors (the extrinsic pathway). Both pathways converge to induce the activation of caspases, executioners of cell death [129]. The link between the caspase signaling cascade and the mitochondria is provided by the Bcl-2 family member Bid. This protein is cleaved by caspase-8 and in its truncated form (tBID) translocates to the mitochondria where it induces the translocation, oligomerization, and insertion of other Bcl-2 family members, which control the integrity of the outer membranes of mitochondria. On the surface of the mitochondrion, Bcl-2 antiapoptotic proteins detect mitochondrial damage and activate two proapoptotic Bcl-2 effector proteins, Bax and Bak (Fig. 16.1). Interactions among these proteins break up the outer mitochondrial membrane and shape channels allowing the release of proteins present in the mitochondrial intermembrane space (such as the cytochrome-c, Smac/Diablo, and apoptosis-inducing factor (AIF)). Once cytochrome c is released, it binds to Apaf-1 to assemble the apoptosome, a complex that triggers the activation of the initiator procaspase-9 [64]. Activation of caspases induces a biochemical cascade, which leads to characteristic changes of cell morphology such as blebbing, shrinkage, nuclear and chromosomal DNA fragmentation, and chromatin condensation [1, 34, 111]. Permeabilization of the outer mitochondrial membrane is antagonized by antiapoptotic proteins such as Bcl-2, Bcl-W, Bcl-xL, A1/Bfl1, and MCL-1, which inhibit Bax and Bak functions. In addition to cytochrome c, other proteins are released to assist or potentiate apoptosis. For instance, Smac/Diablo antagonizes inhibitors of caspases, thereby enhancing caspase activation and apoptosis, and AIF that insures caspase-independent mitochondria-mediated apoptosis inducing chromatin condensation and DNA fragmentation [68].

The mitochondrial permeability transition is a permeability increase of the inner mitochondrial membrane mediated by a channel, the permeability transition pore (PTP) [7, 8]. PTP opening is affected by inducers like calcium and ROS or inhibitors like acidic matrix pH. While short-term opening may participate in physiological regulation of $Ca^{2+}$ and ROS homeostasis, long-lasting opening of the PTP triggers mitochondrial swelling, rupture of the outer membrane, collapse of membrane potential, cessation of ATP synthesis, release of cytochrome c, and other proapoptotic factors which initiate the mitochondrial pathway of apoptosis [8].

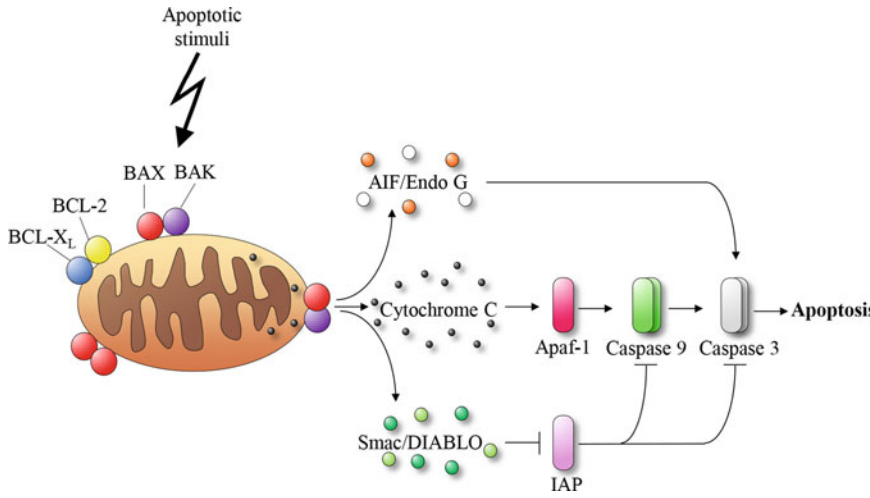


**Fig. 16.1** Scheme of the apoptosis. The proteins of the BCl-2 family control the external mitochondrial membrane integrity. After apoptotic stimuli, BAX and BAK heterodimer break up the outer mitochondrial membrane and opening a channel, which allows the release of proteins present in the mitochondrial intermembrane space such as the cytochrome-c, Smac/DIABLO, and Endo G/AIF. The latter, in turn, led to caspase cascade activation inducing apoptotic modifications. Mitochondrial outer membrane permeabilization is antagonized by the antiapoptotic BCL-2 proteins, such as BCL-2, BCL-W, and BCL-Xl

As mentioned above, mitochondria are the primary intracellular site of oxygen consumption and the major source of ROS, most of them originating from the mitochondrial respiratory chain. In cells with high oxidative capacity as cardiomyocytes, mitochondria are an essential source of ROS and the most direct target for their damaging effects. Cardiomyocyte apoptosis, induced by the overproduction of ROS under ischemia or ischemia/reperfusion (I/R) or ischemic preconditioning (IPC), is an important pathological phenomenon in heart failure (HF). Therefore, a fine equilibrium between ROS production and removal determines the physiological versus pathological function of ROS. In fact, an excessive amount of ROS induces oxidative stress and promotes cell death under hypoxic conditions. Conversely, at physiological levels, ROS function as "redox messengers" in intracellular signaling [22]. For this reason mitochondria contain an arsenal of antioxidant systems with target specificity [68]. ROS can be removed by antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. The first line of defense against ROS is guaranteed by the presence of $Mn^{2+}$-SOD (SOD2) in the mitochondrial matrix, which results in superoxide anion dismutation and the subsequent generation of hydrogen peroxide. Although hydrogen peroxide is not a free radical, it is an oxidant and an intermediate in the chain of reactions that generate reactive free radicals, such as hydroxyl radical, which can oxidize mitochondrial components (proteins, lipids, DNA). Since most mitochondria lack catalase, the metabolism of hydrogen peroxide is mainly accomplished by mitochondrial glutathione, mGSH, with the participation of either GSH peroxidase or peroxiredoxins [22]. It has been demonstrated that a chronic exposure to ROS in the heart will induce apoptosis and fibrosis leading to heart dysfunction and remodeling [85].

Mitochondria play also an essential role in cell calcium homeostasis. They accumulate calcium along its electrochemical gradient, whereas calcium extrusion is an active process involving Na-dependent and Na-independent pathways. Calcium excess leads to uncoupling of oxidation from phosphorylation and mitochondrial membrane depolarization resulting in the opening of the PTP and finally in cell death [123]. The capacity of mitochondria to control excess calcium

depends on the balance between calcium entry along the electrochemical gradient and calcium extrusion mechanisms. This plays a crucial role in cell homeostasis and cell death. Apoptosis is a physiological process, which is necessary for differentiation during embryogenesis. However, apoptosis is also triggered in pathological conditions. In cardiovascular diseases apoptosis may be induced also by mitochondrion-toxic agents. These agents are numerous and include pharmaceuticals, illicit drugs, exotoxins, and food ingredients [15, 63, 75, 103]. Several mitochondrial functions are prone to be affected by toxic agents. The most important among these is the respiratory chain. Dysfunction of the respiratory chain may result in decreased ATP production, increased ROS production, reduced antioxidative capacity, and reduced mitochondrial membrane potential or apoptosis [69]. A number of chemotherapeutic agents are cardiotoxic due to mitochondrial dysfunction [107]. Cardiotoxicity includes acute or chronic cardiovascular complications, which impair quality of life, even years after treatment. Chemotherapeutic agents associated with cardiotoxicity due to mitochondrial dysfunction include anthracyclines (e.g., doxorubicin), anthracenediones (e.g., mitoxantrone), alkylating agents (e.g., cyclophosphamide, cisplatin, ifosfamide, busulfan, mitomycin), vinca alkaloids (e.g., 5-fluorouracil, amsacrine, asparaginase), tyrosine-kinase inhibitors (e.g., imatinib, dasatinib, sunitinib, sorafenib), and other agents (e.g., trastuzumab, paclitaxel, etoposide, teniposide) (Simbre et al. 2005; [34]).

## Mitochondria and Autophagy

Macroautophagy, hereafter referred to as autophagy, is a conserved process aimed at maintaining of cellular and tissue homeostasis under normal as well as stress conditions, including nutrient starvation, changes in metabolism, energy, and oxygen status. Autophagy is a degradation mechanism of cytoplasmic components, including damaged organelles, toxic protein aggregates, and intracellular pathogens [72]. Basal autophagy plays a key role in eukaryotic cells, degrading long half-life macromolecules and large supramolecular structures, including organelles such as mitochondria, peroxisomes, and endoplasmic reticulum [60]. This process is characterized by several steps. Initiation of autophagy describes the formation of the isolation membrane and phagophore, which then expands to engulf the cargo (protein aggregates or damaged organelles), thus forming the autophagosome, a double-membrane intracellular structure of reticular origin. This process is completed by autophagosome clearance, which occurs after fusion with lysosomes, enabling degradation of cargo by lysosomal enzymes [72] (Fig. 16.2). Degradation by-products, such as amino acids, can then be reused for the building of new

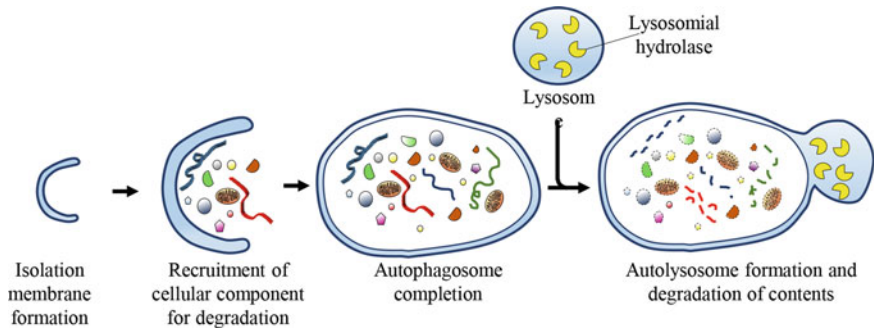


**Fig. 16.2** Scheme of the autophagy process. Phagophore determines the onset of the autophagy. The progressive elongation of the phagophore is accompanied by the recruitment (specific or not) and degradation (autophagosome completion) of cellular components. The outer membrane of the autophagosome may fuse with a lysosome to form autophagolysosome. Finally, the engulfed material is degraded inside the autophagolysosome and recycled

macromolecules or for meeting metabolic demands [54, 127]. Initial phagophore formation requires the assembly of a complex consisting of BECLIN1, vacuolar protein sorting (VPS) 34, and VPS15 [49]. Next, the expansion of the membrane is mediated by two ubiquitin-like conjugation systems, microtubule-associated protein 1 light chain 3 (LC3) and autophagy protein (ATG) 12-ATG5, that promote assembly of the ATG16L complex and the conjugation of LC3 with phosphatidylethanolamine [48, 73]. Autophagy contributes to the maintenance of intracellular homeostasis in most cells of cardiovascular origin, including cardiomyocytes, endothelial cells, and arterial smooth muscle cells.

Mitophagy is an autophagic response that allows elimination of defective mitochondria and accelerates the mitochondrial turnover, thus preserving the pool of healthy organelles [95]. As the mitochondria occupy a critical position in the bioenergetics of the cardiovascular system, mitophagy is particularly important for cardiovascular homeostasis in health and disease [11]. Upstream, among others, two main actors in the regulation of mammalian mitophagy are the serine/threonine kinase PTEN-induced putative kinase 1 (PINK1) and the E3 ubiquitin ligase Parkin, which selectively promote the degradation of impaired mitochondria [78]. Under normal conditions, PINK1 is imported into the mitochondria, where it undergoes rapid cleavage by protease PARL, and maintained at low levels on the inner membrane. When the mitochondria present a decrease of membrane potential, PINK1 accumulates on the outer membrane. In healthy mitochondria, Parkin translocates from cytosol to damaged or uncoupled mitochondria, promoting ubiquitination of several mitochondrial outer membrane proteins, such as MFN1, MFN2, and VDAC. On damaged mitochondria recruitment of Parkin is selective and requires the participation of PINK1 [77]. Ubiquitination of proteins allows recruitment of p62/SQSTM1, an adaptor that interacts with ubiquitinated proteins and LC3, recruiting a phagophore to engulf the ubiquitinated mitochondrion [99]. Although Parkin-induced mitophagy has been shown to be dependent on the activity of PINK1, this process depends on DRP-1-mediated mitochondrial fission [35, 74, 114, 120] (Fig. 16.3). A recent study showed that Parkin mRNA and protein are present at low levels in normal mouse hearts but are upregulated after cardiac myocyte-specific deletion of the *Drp1* gene in adult mice [14]. Thus, *Drp1* deficiency appears to trigger Parkin-dependent over-activation of mitophagy leading to a severe myopathic phenotype. The authors propose that DRP1 helps in the maintaining mitochondrial quality control by promoting mitochondrial fission to segregate dysfunctional mitochondria that can then be targeted by mitophagy [109]. These data highlight the central role of mitochondrial dynamics in cardiac mitochondrial quality control, ensuring proper elimination of damaged and dysfunctional organelles [14].

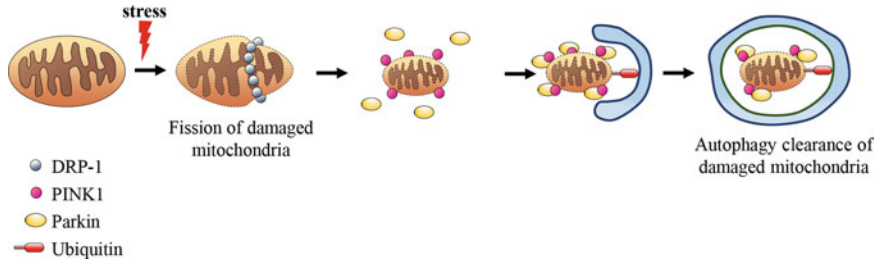


**Fig. 16.3** Scheme of autophagy clearance of damaged mitochondria. Damaged mitochondria (usually with low membrane potential) undergo DRP1-mediated fission to initiate the process of mitophagy. Under reduced mitochondrial membrane potential, PINK1 accumulates on the outer mitochondrial membrane and allows the recruitment of the Parkin E3 ubiquitin ligase. Parkin induces ubiquitination of several surface proteins, which, in turn, trigger the damaged mitochondrion removal by an autophagosome

Mitophagy is also induced by Parkin-independent mechanisms that involve proteins or lipids already present on the outer membrane of the mitochondria. BNIP3 and BNIP3L/Nix, known for their role in apoptosis, interact directly with LC3 to initiate mitochondrial clearance [43, 91]. Fundc1 is a mitochondrial protein located on the outer membrane. It contains a binding domain for LC3 participating in mitochondrial engulfment. These pathways are triggered by different stimuli. As already mentioned, Parkin-PINK1 pathway is induced by a decrease in membrane potential, while Fundc1 has been reported to play an important role in the response to hypoxic stress [62]. All of them may be involved in cardiac stress [38]. Indeed, Parkin translocation to the mitochondria takes place during I/R [56]. It has been suggested that infarction-induced mitophagy is a beneficial homeostatic response to protect the heart [29]. Bnip3 is also activated during I/R and triggers mitophagy [57]

## Mitochondria and Senescence

Cellular senescence is an irreversible growth arrest accompanied by inability to repair tissue damages occurring in response to various cellular stimuli, such as telomere erosion, DNA damage, and oxidative stress. Senescence is associated with the appearance of several biomarkers such as β-galactosidase activity and p53 activation [5, 12]. In model organisms, senescence is accompanied by mitochondrial malfunctions, which subtend the observed age-dependent decline in organ function [96]. Similarly, defects in mitochondrial function have also been observed in human. Moreover, some mitochondrial damages may predispose humans to certain age-related diseases. As a result, chronic diseases, including cardiovascular diseases, increase their prevalence with aging. Cardiac aging is characterized by the presence of hypertrophy, fibrosis, and defect in contractility, calcium handling, cell metabolism, and mitochondrial function [55]. The aging heart is generally associated with decreased protein quality control and dysfunctional mitochondria [106, 116].

Increased production of ROS emerged as the main proximate cause of aging. Mitochondria have been involved as key players, first because they are an important source of free radicals, especially in highly oxidative tissues, and second because they are a direct target of oxidative damage in aging cells [123]. As age increase, ROS tend to raise their contents as a result of the accumulation of damages to mitochondrial proteins, an imbalance between oxidative stress and antioxidant mechanisms, and declines in activity of mitochondrial respiratory chain complexes. Considering this, ROS regulate senescence diseases [86, 116]. There is increasing evidence that mitochondria can regulate cellular aging through the modulation of the metabolic profile of the cell [106, 113]. The key role of ROS generation and mitochondrial dysfunction in cardiac aging is supported by experiments that target mitochondrial ROS. For example, overexpression of the ROS scavenger enzyme catalase attenuates the development of hypertrophy, fibrosis, and diastolic dysfunction in the aging mouse heart [25, 51]. By contrast, prematurely aging mice with a mutation in mitochondrial DNA polymerase exhibit marked cardiac hypertrophy and fibrosis as well as systolic and diastolic dysfunction [26, 51]. Together, these observations suggest that ROS generation and mitochondrial damage contribute to cardiac aging.

ROS production by mitochondria may also be a significant mechanism by which cardiovascular risk factors lead to the formation of vascular lesions in a sex-specific manner [111]. Vascular diseases are instrumental in aging, as well as cardiac and neurological disorders.

## Fusion and Fission of Mitochondria

Mitochondria are present in all cells except for erythrocytes. Depending on a tissue's function, mitochondria may be more or less prominent within a cell type. In the heart about 45% of

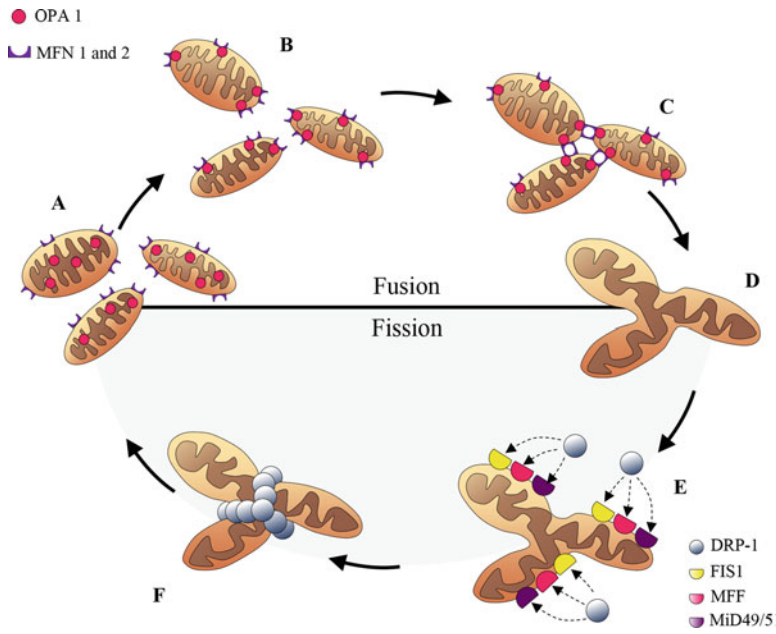


**Fig. 16.4** Mitochondrial fusion and fission processes subtending the cell homeostasis (*A–D*) *Mitochondrial fusion*. The fusion process is driven by mitofusins (MFN1 and 2) and OPA1 usually located on the outer and inner mitochondrial membranes, respectively. Fused mitochondria both share materials (matrix components, damaged mitochondrial DNA) and bioenergetic properties (e.g., mitochondrial membrane potential) (*D–F*) *Mitochondrial fission*. DRP1 bind to FIS1, MFF, and MiD49/51 adapters starting to the moiety separation (fission)

the myocardial volume is taken up by mitochondria [31].

Interestingly, morphology of mitochondria differs among different cell types: for example, solitary organelles are retrieved in hepatocytes, whereas in many epithelial cells, they form an intricate network [23]. In adult cardiac myocytes, mitochondria localize within three subcellular distributions: interfibrillar, subsarcolemmal, and perinuclear. The mitochondrial morphology is mostly globular in the perinuclear region and predominantly rod shaped in the subsarcolemmal space, while inter-myofibrillar mitochondria have the same size of a sarcomere [31].

Ultrastructure and morphology of mitochondria are continuously regulated by fusion and fission balance events [6] (Fig. 16.4). Mitochondrial fusion is a complex sequential process, which involves integration of the outer mitochondrial membrane, inner mitochondrial membrane, and matrix content. The main regulators of these processes are the GTPase dynamin-related proteins: mitofusin 1 (MFN1) and mitofusin 2 (MFN 2), located in the outer mitochondrial membrane, and optical atrophy 1 (OPA1), located in the inner mitochondrial membrane [120]. MFN1 and MFN 2 are both needed for mitochondrial *fusion. In fact, their* loss results in a reduction of this process. Moreover, these proteins are partially redundant in their function; thus when MFN1 expression is decreased, mitochondrial fusion can be supported by MFN2 overexpression and vice versa [17]. Additionally, MFN2 has been implicated in several other physiological functions, such as modulation of energetic processes, endoplasmic reticulum-mitochondria coupling, and regulation of mitophagy, a process through which mitochondria are engulfed by autophagosomes and delivered to lysosomes for degradation [18, 120]. OPA1 is a transmembrane protein tightly associated with the mitochondrial inner membrane. The transcript coding for OPA1 is subjected to alternative splicing, giving rise to eight variants that are expressed in a variety of patterns across different tissues. In particular, both OPA1 short (88 kDa) and long (112 kDa) isoforms are necessary for mitochondrial fusion [108].

The opposite process (mitochondrial fission) results from mitochondria fragmentation and is regulated by the large GTPase dynamin-related protein (DRP-1), mitochondrial fission 1 (FIS1), mitochondrial fission factor (MIFF), and mitochondrial dynamic proteins of 49 and 51 kDa

(MiD49/51) [42, 97]. DRP-1 is a cytosolic protein, which lacks a mitochondrial destination sequence. It requires localization of FIS1 in the mitochondrial outer membrane to form the fission complex [10]. DRP1, like dynamins, functions as a mechanoenzyme, serving to constrict the mitochondrion physically, an early step in fission. However, in mammalian cells silencing FIS1 has little effect on DRP1 translocation to mitochondria [58]. MFF appears to be the protein acting as the DRP1 mitochondrial receptor. Reduction of MFF levels induces mitochondrial elongation a decrease of DRP1 translocation to mitochondria [36]. Likewise, MiD49 and MiD51 are involved in the mammalian fission machinery [81].

These processes play a central role in mitochondria quality control and are important for maintaining various cellular functions and viability. Fusion and fission balance is very important for mitochondrial participation in crucial cellular processes. The first is a necessary adaptation to nutrient starvation and increased metabolic demand [92]. The latter is required for mitochondrial proliferation following mitosis [50], apoptosis [128], and removing damaged mitochondria from the cells through mitophagy [118]. Mitochondrial fission is essential for maintenance and repair. It facilitates the removal of damaged components by partitioning them, so they can be targeted for removal and degradation by mitophagy. However, excessive mitochondrial fission and mitophagy can compromise the metabolic capacity of a cell [119]. Therefore, it is necessary to maintain an adequate equilibrium in the fission/fusion cycle. The connection between these processes and the onset of heart disease is not yet entirely clear. Although mitochondrial fusion and fission are most evident in neonatal cardiac myocytes, there is strong evidence that these processes are important in the adult cardiac tissue. In fact, the proteins involved in fusion and fission are highly expressed in the adult heart [30, 101]. More evidences for the importance of mitochondrial dynamics in the heart result from studies on the loss of functions of proteins involved in this process. For example, in cardiac myocytes isolated from murine models of an inducible ablation of MFN-1/2, fragmented mitochondria with abnormal cristae have been detected [83]. These morphological changes were accompanied by alterations in mitochondrial respiration, mitophagy, and mitochondrial biogenesis, which culminated in cardiomyopathy [21, 84]. Abnormalities in mitochondrial morphology were also observed in mice heterozygous for OPA1. These mitochondria, large and with abnormal cristae, manifest increased ability to accumulate $Ca^{2+}$ and are marked by a delayed opening of the mitochondrial PTP coupled with increased sensitivity to prolonged mechanical stress. The decreased expression of OPA1 did not alter mitochondrial respiration [88]. Chen et al. described small and fragmented mitochondria in both human and rat models of heart failure, which were associated with decreased OPA1 levels [19, 20]. Decreased levels of Mfn2 mRNA were detected in neonatal rat cardiac myocytes exposed to phenylephrine to induce hypertrophy and in vivo models of cardiac hypertrophy [32]. Similar results were reported by Papanicolaou et al. They found that MFN2-deficient mice display modest cardiac hypertrophy accompanied by a slight functional deterioration [83]. Ong and coworkers found that inhibition of DRP1 increases the proportion of adult cardiac myocytes with elongated mitochondria and protects them against simulated I/R [79]. Moreover, Sharp et al. reported that DRP1 inhibition has therapeutic benefits even when administered after ischemia [104].

There are many evidences of the role played by the mitochondrial dynamics on vascular smooth muscle cells (VSMCs) and its effect on vascular diseases, which are instrumental for cardiac disorders. The principal function of VSMCs is the regulation of vascular tone and, consequently, blood pressure and blood flow. Unlike cardiac myocytes, VSMCs are highly plastic and undergo reversible changes in phenotype in response to environmental stimuli [46, 67]. When a vessel is damaged, these cells, which differentiated have a contractile phenotype characterized by little proliferation, proliferate and migrate toward the injury site [47]. Recently, a relationship has been proposed between the

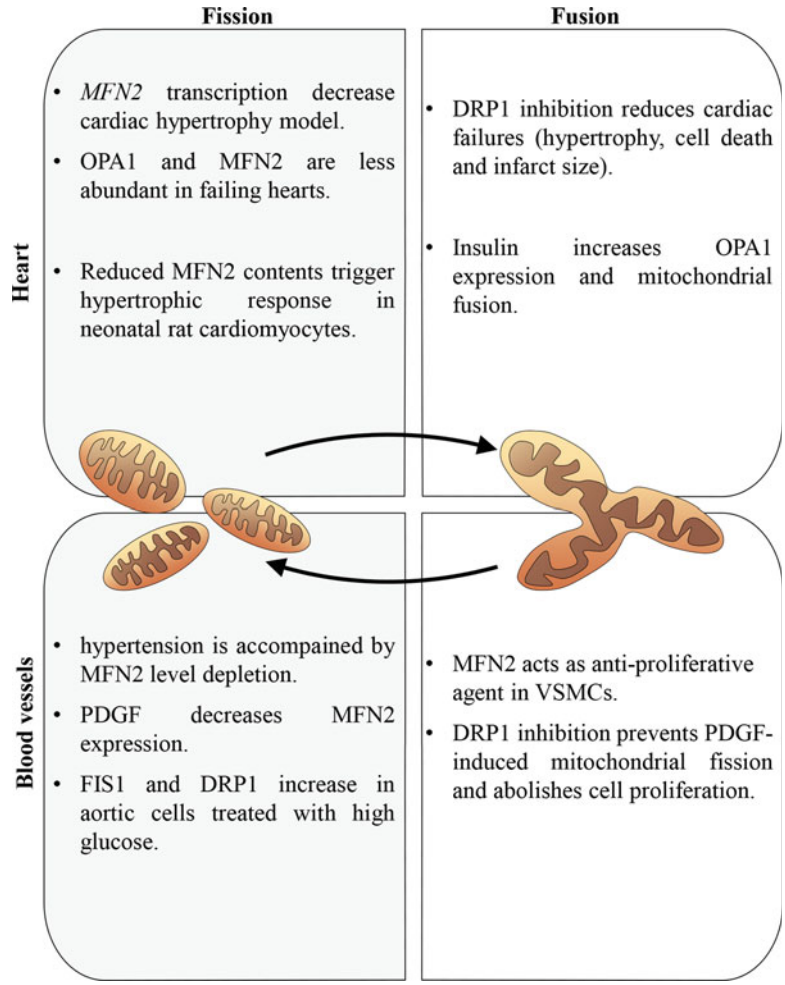


**Fig. 16.5** Mitochondrial dynamics and cardiovascular diseases Summary of major molecular events related to the mitochondrial fission and fusion in cardiovascular diseases

VSMC proliferative phenotype and mitochondrial dynamics [16, 65]. VSMC proliferation can be induced by several factors, including platelet-derived growth factor (PDGF), insulin-like growth factor 1 (IGF-1), angiotensin II, and endothelin-1 [45, 90, 125]. Lately, it has been shown that PDGF induces mitochondrial fragmentation reducing MFN2 levels [100]. Shenouda et al. reported mitochondrial fragmentation and increased levels of FIS1 and DRP1 proteins in cultured human aortic endothelial cells incubated with high glucose medium [105]. Figure 16.5 summarizes the effects of proteins involved in fusion-fission mechanism on cardiovascular disease [120].

## Mitochondria and Gender Difference

It is well known that gender affects several health issues. Women are more susceptible than men to depression, osteoporosis, asthma, smoke-induced lung cancer, and autoimmune diseases. However, not all medical problems show gender dimorphism. For example, males do not differ from females in infection responses [59]. Both clinical and experimental observations show that also in cardiovascular diseases, gender differences play a key role. The incidence of cardiovascular diseases increases with age in both sexes, although men and women are predisposed toward different

cardiovascular diseases with old age [33, 40, 51]. Male/female differences in coronary artery disease, including a higher risk of obstructive disease in men and more microvascular disease in women [41], clearly contribute to sex differences in cardiovascular disease expression.

Men undergo higher health risks (ischemic heart diseases, hypertension, arrhythmias, and heart failure) than age-matched premenopausal women. This data, together with the observation that incidence of cardiovascular diseases tends to increases in women after menopause, support the notion that the incidence of cardiovascular diseases is associated with the decreasing levels of estrogens during menopause [93].

It has been shown that cardiomyocytes from female rats exhibit lower mitochondrial content, but female mitochondria are more efficient, more differentiated, and generate less ROS than the male ones [123].

As said above, mitochondria are particularly abundant in the heart, and their content and function are sex-dependent. It has been reported that the female cardiac muscle generates less mitochondrial ROS and exhibits lower oxidative damage than those of males. Several studies demonstrate that cardiovascular diseases such as myocardial infarction and atherosclerosis are underrepresented in premenopausal women compared to their male counterparts [4]. Although the mechanisms underlying sexual dimorphism in disorder development are uncertain, sex differences in the levels of biomarkers responding to oxidative stress have been observed in clinical and experimental studies. It has been shown that oxidative stress biomarkers are lower in healthy young women than in age-matched men [44, 89]. Moreover, studies on rat model systems shown that male produced more ROS than age-matched females [24].

Significant gender differences were also found in the uptake of $Ca^{2+}$ by cardiac mitochondria [3]. Mitochondria from female rat heart showed lower $Ca^{2+}$ uptake rates in physiological substrate solutions and maintained mitochondrial membrane potential in presence of $Ca^{2+}$ at high dosage. Since mitochondrial calcium overload is an important factor in defining cardiac I/R injury, these differences may be explain why female myocardium suffers less injury with I/R [80, 123].

It is widely accepted that mitochondrial dysfunction, and particularly mitochondrial PTP opening, plays a major role in determining the extent of cardiac I/R injury. Recently, it has been proposed that the increased resistance of female heart mitochondria reflects regulation of mitochondrial PTP function rather than changes in the putative components [71]. In this context, sex hormones could be responsible for these effects because they have been reported to directly regulate several mediators of the mitochondrial biogenesis program. Regulation of mitochondrial function and biogenesis by estrogens/estrogen receptors has been extensively reviewed [19, 20, 53, 87]. Estrogen receptor may bind to mtDNA and is involved in the E2-induced expression of mtDNA and respiratory chain proteins. Several studies demonstrated that sex differences in oxidative stress markers are estrogen-dependent and that estrogen may exert protection in females by increasing antioxidant defenses and by diminishing ROS production [124]. Interestingly, a cross talk between mitochondrial function and sex-steroidal hormones during aging is reported [121]. Finally, estrogen may be considered a key factor subtending sex differences in mitochondrial function and morphology, ROS production, and antioxidant activity. In fact, estrogen by its receptor can upregulate the expression of NRF1, a key transcription factor regulating transcription of majority of mitochondrial respiratory chain complexes. Moreover, estrogen can also modulate the transcription of NRF1 indirectly through interaction with another transcription factor, PGC-1 [52].

Literature data confirm that premenopausal women experience less cardiovascular diseases compared with age-matched men. However, in postmenopausal women the rate of cardiovascular disease development and mortality from cardiac disease exceeds those of men. This corroborates the key role played by estrogens. Considering sex differences in the development of HF, it was shown that in hearts of female mice subjected to pressure overload, downregulation of metabolic

and mitochondrial biogenesis transcription cascade genes are less important than in male hearts [126]. These differences could contribute to the protection of females against HF.

Sex-related specificity has also been found in HF induced by toxic agents like anthracyclines. Anticancer therapies involving anthracyclines are limited by their cardiotoxicity. His cardiotoxicity is considered as a complex multifactorial process involving oxidative stress and mitochondrial damage [112, 115]. It has been demonstrated that female rats seem much less sensitive to the cardiotoxic effects of doxorubicin (the main anthracycline) than male rats [37, 76].

Also in ischemic heart disease, the leading cause of morbidity and mortality in both men and women, women have lower risk before menopause [80, 94]. Ischemia and postischemic reperfusion cause a wide array of functional and structural injuries to mitochondria, due in part to excess production of ROS and calcium overload. This triggers PTP opening, decrease in ATP supply, and ultimately cell death [8]. Heart from female rat is more resistant to oxygen deprivation [9], and ischemic reperfusion injury induces lower infarct size in female than male rats [70]. A similar observation was made in patients with acute myocardial infarction. Primary percutaneous coronary intervention results in better myocardial salvage in women than men [70].

Sex-dependent ROS production by mitochondria may also be a significant mechanism by which cardiovascular risk factors lead to the formation of vascular lesions in a sex-specific manner [111].

Other sex differences may be revealed in response to cell stress. Exposure of rat VSMCs to ultraviolet radiation induces an upregulation of survival proteins in cells from females and an increased proapoptotic proteins and loss of mitochondrial membrane potential in cells from males [66]. Furthermore, cells from female rats show adhesion-associated resistance to apoptosis, which is apparently due to a more adhering phenotype, characterized by a higher propensity to undergo survival by autophagy [110].

Summing clearly, part of the protective effect of female sex in CVDs is linked to (i) better protection of mitochondrial function and content in female heart and vessels and (ii) better ability to handle calcium and to decrease ROS production.

## Conclusions

Mitochondria are pivotal organelles for cell fate. Their involvement in cardiovascular diseases is increasingly acknowledged. Alterations of mitochondrial mass and function play an important role in CVDs. Particularly, can be hypothesized that a different mitochondrial function could be responsible for gender differences in CVDs. Future research should consider both sexes, not only to better understand the pathophysiology of the diseases but also to suggest more appropriate therapeutic interventions.

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