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## **Mitochondria: a central target for sex differences in pathologies**

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### ABSTRACT

It is increasingly acknowledged that a sex and gender specificity affects the occurrence, development and consequence of a plethora of pathologies. Mitochondria are considered as the powerhouse of the cell because they produce the majority of energy-rich phosphate bounds in the form of **adenosine tri-phosphate (ATP)** but they also participate in many other functions like steroid hormone synthesis, ROS production, ionic regulation and cell death. Adequate cellular energy supply and survival depend on mitochondrial **life cycle, a process involving mitochondrial biogenesis, dynamics, and quality control via mitophagy.** It appears that mitochondria are the place of marked sexual dimorphism involving mainly oxidative capacities, calcium handling, and resistance to oxidative stress. In turn, sex hormones regulate mitochondrial function and biogenesis. Mutations in genes encoding mitochondrial proteins are at the origin of serious mitochondrial genetic diseases. Mitochondrial dysfunction is also an important parameter for a large panel of pathologies including neuromuscular disorders, encephalopathies, cardiovascular diseases, metabolic disorders, neuropathies, renal dysfunction etc... Many of these pathologies present sex/gender specificity. Here we review the sexual dimorphism of mitochondria from different tissues and how this dimorphism takes part in the sex-specificity of important pathologies mainly cardiovascular diseases and neurological disorders **(Figure 1).**

**Keywords:** mitochondria, sex, cardiovascular diseases, neurological disorders, sexual dimorphism, gender, metabolic diseases, oxidative stress, aging.

### INTRODUCTION

Sexual dimorphism in anatomical, physiological and behavioral traits is a characteristic of mammalian species and results from genetic, epigenetic, hormonal and environmental factors. **In almost every country where records have been kept, differences in human life expectancy between men and women can be observed (1).** Sexual differences in the prevalence and manifestations of many human diseases have been described, although these aspects are marginally taken into account and their biological basis are still far from being elucidated (2-5). For example, many epidemiological studies have demonstrated that premenopausal women have a reduced risk of cardiovascular and neurological diseases compared to their male counterparts and that in postmenopausal women the risk reaches or even exceed the rate for men (6-8). This arises from sex- and gender-differences. In medical research, sex refers to biological differences while gender refers to psychosocial and cultural differences between men and women, which may impact on risk factors, medical diagnosis and therapeutic management (9). We will focus here on sex-dependent pathological differences.

Mitochondrial dysfunction has been associated with a large variety of human disorders, such as cardiovascular and neurodegenerative diseases, aging, toxicity of drugs and others (10-12). Aside from providing most of the energy to the host cell, mitochondria participate in multiple other cell functions. Mitochondrial bioenergetics and oxidative stress have been involved in sex dimorphism and in sex differences in the occurrence and the development of numerous pathologies, as well as the sensitivity to therapeutics.

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Here we review the sexual dimorphism of mitochondrial structure and function in different tissues. We present evidence for the involvement of mitochondria in the sex-specificity of aging and diseases from examples mainly taken from cardiovascular and neurological disorders.

### WHY ARE MITOCHONDRIA UNIQUE

#### a. Mitochondrial life cycle

The first unique feature of mitochondria is that they arose through an endosymbiotic process more than 1.45 billion years ago. The second is that they are exclusively maternally inherited. In most eukaryotic cells, energy metabolism is mainly aerobic. Aerobic metabolism takes place in these specific endosymbiotic organelles, the mitochondria, having their own circular DNA and being present in variable number and shape in cells. They consist in an external membrane and an inner membrane formed by multiple invaginations or cristae connected to the boundary region of the inner membrane at narrow circular junctions (13), delineating a matrix and an intermembrane space. Mitochondria contain their own circular DNA (mtDNA) and the human mitochondrial genome encodes 13 subunits of the mitochondrial respiratory chain, 2 ribosomal and 22 transfer RNAs. In mammals, the mitochondrial proteome contains more than 1000 proteins, not counting a wide array of splicing and post-translational variants (14). Because most of the mitochondrial proteins are encoded by the nuclear genome, mitochondria have tissue-specific structure and function rendering them exquisitely well-adapted to the physiology of the host cell. They thus exhibit a large diversity in structure, biochemistry and function. Each cell contains multiple mitochondria (7000-10000 in cardiac cells) and their number and activity are highly dependent on the cell type and energy requirements (15, 16).

Adequate cellular energy supply and survival depend on mitochondrial life cycle, which involves mitochondrial biogenesis, dynamics, and recycling via mitophagy (Figure 2). Mitochondria proliferate by division of preexisting organelles, through a process called mitochondrial biogenesis. Mitochondrial biogenesis is under the control of the nucleus and necessitates the coordination of the two genomes, the nuclear and the mitochondrial ones. The nuclear-encoded mitochondrial transcription factor A (TFAM) activates the transcription and the replication of the mitochondrial genome. In turn, TFAM transcription is activated by the nuclear respiratory factors (NRF) 1 and 2 and the peroxisome proliferator-activated receptor gamma, coactivators 1 (PGC-1 $\alpha$  or  $\beta$ ), the master regulators of mitochondrial biogenesis (17). These co-activators 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. The estrogen-related receptor family of transcription factors (ERR $\alpha$ ,  $\beta$ ,  $\gamma$ ), as well as the peroxisome proliferator-activated receptors (PPAR $\alpha$ ,  $\beta$ ,  $\delta$ ) are also involved in mitochondrial biogenesis and expression of metabolic genes.

An important step in mitochondrial life cycle is the fission of the mother mitochondria into two daughter mitochondria, which is coordinated by specialized GTPases. In most cells mitochondria are present as a network undergoing frequent fusion and fission events. This process called mitochondrial dynamics is an important factor in several disease conditions. Fission is driven by dynamin-related protein 1 (DRP1) and its docking proteins, fission protein 1 (Fis1) and mitochondrial fission factor (Mff). Fusion is controlled by mitofusins (Mfn1 and 2) for the outer membrane and OPA1 for the inner membrane (18). The final process of

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mitochondrial biogenesis needs the biosynthesis of membrane lipids among which the inner-membrane-specific phospholipid, cardiolipin (19). Finally, poorly functioning mitochondria can be eliminated through the process of mitochondrial autophagy (mitophagy), which not only allow elimination of defective mitochondria but also may accelerate the mitochondrial turnover, thus preserving the pool of healthy organelles (20). Mitophagy refers to the targeted lysosomal degradation of organelles for metabolic recycling.

Upstream, among others, two signaling pathways activated by the energetic status can activate mitochondrial biogenesis: the AMP-activated protein kinase (AMPK) which senses the energetic state through the AMP/ADP over ATP ratio and the sirtuin 1 (SIRT1) which senses the redox state through the NAD/NADH ratio (21).

### b. Mitochondrial functions

Mitochondria are the site of oxidative phosphorylations (OXPHOS). They are considered as the powerhouse of the cell because they produce the majority of energy rich bounds in the form of ATP (>90% for the heart). They use mainly fatty acids and carbohydrate-deriving substrates to produce reducing equivalents that are oxidized along the respiratory chain, providing a proton gradient across the inner membrane that is used as a driving force to produce ATP from ADP.

Aside from providing most of the energy to the host cell, mitochondria participate in multiple other cell functions like ionic homeostasis, production and regulation of ROS, pH regulation, steroid hormones synthesis, calcium homeostasis, thermogenesis, lipid and carbohydrate utilization, and cell death.

Importantly, a by-product of the electron transport is the generation of reactive oxygen species (ROS), essentially at complex I and III of the respiratory chain. ROS include the superoxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), and hydroxyl radicals ( $OH^\bullet$ ). ROS levels are tightly regulated via a range of antioxidant defenses including enzymatic reactions like mitochondrial superoxide dismutase (SOD), catalase, glutathione peroxidase and reductase as well as non-enzymatic reactions. Complex I and III generate superoxide radicals which are converted to  $H_2O_2$  by SOD.  $H_2O_2$  can be degraded to water and oxygen by catalase or transformed into  $OH^\bullet$  (rather active ROS) in the Fenton reaction. Naturally low levels of ROS represent key signaling molecules to regulate biological and physiological processes (22). However, when mitochondria are dysfunctional, higher production of ROS can cause irreversible damages to lipids, DNA and proteins.

Mitochondria are instrumental for cell life and death. The mitochondrial permeability transition is a permeability increase of the inner mitochondrial membrane mediated by a channel, the permeability transition pore (PTP) (see (23, 24) for detailed description and mechanistic). PTP opening is affected by inducers like calcium and ROS or inhibitors like adenine nucleotides and 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 outer membrane, collapse of membrane potential, cessation of oxidative phosphorylation and ATP synthesis, release of cytochrome c and other pro-apoptotic factors which initiate the mitochondrial pathway of apoptosis (23).

Mitochondria also play an essential role in cell calcium homeostasis. They are in close spatial interactions with the main calcium stores, the endo/sarcoplasmic reticulum. Mitochondria accumulate calcium along its electrochemical gradient whereas calcium extrusion is an active process involving Na-dependent and independent pathways. Calcium accumulation may favor oxidative phosphorylation by stimulating several mitochondrial dehydrogenases, but excess calcium leads to uncoupling of oxidation from phosphorylation and mitochondrial

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membrane depolarization resulting *in fine* in opening of the PTP and cell death (23).

Mitochondria play an essential role in sex steroid hormone biosynthesis and in turn these sex steroid hormones like estrogen, progesterone and testosterone may regulate mitochondrial function (25). Cells contain functional estrogen (ER), androgen (AR) and progesterone (PR) receptors that are members of the steroid hormone receptor family of ligand-activated transcription factors and are targets for sex hormone action. Two ERs, ER $\alpha$  and ER $\beta$  have been described and additional G-protein-Coupled membrane receptors (GPER). Estrogens (E2) may have genomic and non-genomic effects. The genomic effects involve binding of hormone-bound receptors on hormone responsive elements and regulation of the expression of specific genes. Non genomic effects involve rapid, within seconds or minutes, signaling effects through activation of the membrane-associated ERs. In addition to their presence in nuclei and plasma membrane, ER and AR are also localized in mitochondria of a number of cell types and tissues (reviewed in (26-28)). In mitochondria, ERs are bound to mtDNA, suggesting hormonal action on mtDNA transcription and replication. Estrogens can increase the expression of mitochondrial proteins from both nuclear and mitochondrial genomes and favor mitochondrial biogenesis (27, 29). Estrogen regulation of mitochondrial capacity and function has been shown to participate in vascular, cardiac and neuronal protection (25, 30-32). Effect of androgens on mitochondrial function and capacity is less clear.

## SEXUAL DIMORPHISM OF MITOCHONDRIA

Mitochondria exhibit a strong tissue- and sex- specificity which is summarized in Table 1.

The sexual dimorphism of biological processes includes differences in sex chromosomes, in exposition to hormones in early life and adulthood, and in epigenetic mechanisms. Mitochondria exhibit a strong sex-specific behavior as they are exclusively maternally inherited and exert differential effects in males and females. Because of this exclusive maternal transmission, the interest in the role of mitochondria in sex determination is growing. The genetic materials in the mitochondrial genome and in the X chromosome are asymmetrically inherited in mammals. Through evolution these genes spend relatively more time under selection in females and are therefore expected to be better optimized for function in females than in males (33). There is a reciprocal link between mitochondria and genetic regulation. It has been hypothesized for example that interaction between sex determination and mitochondria would mean that male sex is determined by nuclear genes inherited from the father regulating the activity of maternally derived mitochondria (34). Mitochondrial function extends to genetic interaction between sex chromosomes and autosomes. It has been suggested that the crosstalk between the Y chromosome and autosomes may be further mediated by the mitochondria (35).

### a. Substantial sex differences in mitochondrial respiratory function

A sexual dimorphism of mitochondrial oxidative capacities has been described in many tissues. In liver, the ADP-stimulated respiration, protein content and cardiolipin levels are higher in female than male mitochondria (36-38). In large rat cerebral arteries, basal and maximal respiration, ATP production, proton leak, and spare respiratory capacity are elevated in female compared to male mitochondria (39). In mouse brain, mitochondria of young females have higher NADH-linked respiration compared to males, independently of estrus cycle (40). Mitochondria from brain of female rodents have higher electron transport

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chain activity and ATP production (41) and greater functional capacities (42). This higher specific activity of female mitochondria from rodent brain was also found in humans. Examining the mitochondrial enzyme activities in post mortem human brains, Harish et al observed that the activities of citrate synthase, succinate dehydrogenase and mitochondrial reductase (MTT) were significantly higher in female compared to male brains (43).

Sexual dimorphism in mitochondrial functionality has also been found in rat white adipose tissue (44), brown adipose tissue (45, 46) and skeletal muscle (47), with females presenting more functional mitochondria than males. Skeletal muscle mitochondria from female rats have higher mitochondrial DNA and protein contents, TFAM protein level, oxidative and phosphorylative machinery and activities, and glutathione peroxidase activity than male ones (48). In human adipose tissue, expression of genes involved in mitochondrial function is higher in women than men (49).

Cardiomyocytes from female rats exhibit lower mitochondrial content, but female mitochondria are more efficient and more differentiated than the male ones (50). At baseline, no difference in oxygen consumption rate and cardiolipin content is observed between mitochondria from male or female rats (51). Sub-sarcolemmal and intermyofibrillar isolated mitochondria from female hearts have the same respiration rates as the male ones except for glutamate-malate stimulated respiration which is lower in females rats while ADP/O ratio is higher (52). Taken together these results suggest that cardiac mitochondria from females have higher specific activity than the male ones but lower mitochondrial content, explaining the similar oxidative capacity in males and females.

Mitochondria rely on two main substrate sources, carbohydrates and lipids. Skeletal muscles of women have higher intracellular lipid content as compared with men due mainly to a greater number of lipid droplets (53). Preferential utilization of fatty acids was observed in female mouse hearts during exercise (54) compared to males. While female mitochondria from rat brain preferentially rely on lipids, male mitochondria utilize mainly proteins (12). Thus, in general female mitochondria preferentially utilize lipids and exhibit higher oxidative capacity than the male ones.

It is well established that female rodents have much higher endurance capacities than their male counterparts (55). Endurance capacity is highly related to skeletal muscle mitochondrial content (56). Compared to males, gastrocnemius muscle of female rats shows higher mitochondrial DNA and protein content, TFAM protein level, oxidative and phosphorylative machinery and activities, and glutathione peroxidase activity (48). This substantiates a clear sex dimorphism in muscle mitochondrial features, which could explain the higher facility of females to adapt to altered metabolic energy situations.

### b. Sexual dimorphism and oxidative stress

In cells with high oxidative capacity like cardiomyocytes, skeletal muscle cells and neurons, mitochondria are an essential source of ROS and the most direct target for their damaging effects. ROS affect the mitochondrial machinery and chronic ROS exposure induces mtDNA mutations that accumulate with age. A sexual dimorphism in the mitochondrial generation of ROS has been widely demonstrated. The redox feature of the cell from a gender perspective has been extensively reviewed (57).

Oxidative stress specially affects the brain mitochondrial function. Brain mitochondria from female rats have less oxidative damages than males whatever the age (58). In mouse brain, lower oxidative stress was found in mitochondria of young females compared to males, independently of estrus cycle (40). However, the brain mitochondria from young male mice



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have a better glutathione cycle than female mice, a difference that disappears at 10 months of age (41).

Mitochondria from female **rat** liver produce half the amount of peroxides than those of males and have a higher content of antioxidant enzymes (59, 60). This is due to higher expression and activities of Mn-SOD and of glutathione peroxidase in females, leading to lower oxidative damage to key mitochondrial components in females than in males (60). Cardiomyocytes from female rats exhibit lower mitochondrial content, but mitochondria are more efficient and generate less ROS than the male ones (50, 61). Estrogen receptors and their signalling cascade are implicated in the control of anti-oxidant enzyme expression (60). The enhanced mitochondrial function and lower oxidative stress in **rat** cardiac muscle, seems to be mediated through G-protein coupled estrogen receptors (62).

Thus at least in liver, brain and heart, female mitochondria produce less ROS and have higher capacity of antioxidant defenses than the male ones (57).

### c. Calcium sensitivity and opening of the mPTP

Oxidative stress and calcium handling by mitochondria are key players in cell fate. The occurrence of a  $\text{Ca}^{2+}$ - and ROS-dependent PTP opening, leading to mitochondrial swelling and its detrimental consequences on ATP synthesis, plays a crucial role in cell homeostasis and cell death. The capacity of mitochondria to control excess calcium is dependent on the balance between calcium entry along the electrochemical gradient and calcium extrusion mechanisms.

In mouse brain, calcium uptake capacity is lower in female than male mitochondria (63). Studying the tissue and sex specificities in  $\text{Ca}^{2+}$  handling by isolated mitochondria, it was shown in **rat** that calcium kinetics differ between male and female mitochondria from **heart only** (37). A greater mitochondrial calcium retention capacity was observed in cardiac mitochondria of female rats compared to males (52). Mitochondrial calcium overload is an important factor in defining cardiac ischemia/reperfusion injury. Female **rat** cardiac mitochondria are more resistant both in the extent and in the rate of the mitochondrial swelling at high calcium concentration (64). The lower  $\text{Ca}^{2+}$  uptake rates and the maintenance of membrane potential under conditions of high  $\text{Ca}^{2+}$  in female **rat** cardiac mitochondria depends on modulation of the calcium uniporter (65). At physiological concentrations, estrogens protect heart mitochondria from high calcium-induced release of cytochrome c (66).

Thus mitochondria from females seem to better tolerate calcium overload, which may contribute to the better tolerance to ischemia/reperfusion injury.

### d. Estrogens and estrogen receptors activate mitochondrial biogenesis in a tissue specific manner

Regulation of mitochondrial function and biogenesis by estrogens/estrogen receptors has been extensively reviewed (27, 29). ER may bind to mitochondrial DNA and is involved in the E2-induced expression of mtDNA and respiratory chain proteins (**Figure 3**). Estrogens increase expression of the master regulator of energy metabolism and mitochondrial biogenesis PGC-1 $\alpha$  and of its downstream targets (67). This is mediated by ER $\alpha$  and by the presence of an estrogen responsive element (ERE) in the promoter of the NRFs (68). E2 stimulates the interaction of ERs with TFAM and other factors in mitochondria and stimulates mtDNA transcription. This effect may be tissue-specific. Fernández-Vizarra et al have investigated sex- and tissue-specificity of the OXPHOS system in rats. They showed that



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the amount of mtDNA/nDNA differs in a sex and tissue-specific manner. It was significantly higher in **rat** female liver and skeletal muscle but similar between males and females in heart tissue (16).

A significant sexual dimorphism in expression levels of cardiac mitochondria-related genes was observed in young, adult and old rats with increased expression of genes of fatty acid metabolism in young females and higher expression levels of genes associated with oxidative phosphorylation in old female hearts compared to males (69). Estrogens increase PGC-1 $\alpha$  expression in the heart following trauma haemorrhage (70).

In the **mouse brain**, the expression of key regulators of mitochondrial biogenesis is sex dependent. For example expression of PGC-1 $\alpha$ , AMPK $\gamma$ 1, Sirt1, NRF2 and Mfn2 is lower in females compared to males, while in the kidneys expression of AMPK and Sirt1 only is lower in females (71). E2 stimulates mitochondrial biogenesis, decreases ROS production and stabilizes mitochondrial structure in **rat** white and brown adipocytes, cardiomyocytes, and skeletal muscle cells (32, 44-46, 48, 72). In turn, PGC-1 $\alpha$  is a coactivator of ER $\alpha$ -dependent transcriptional activity (73).

Estrogens exert protective effects on mitochondria of brain vasculature. In **human** brain endothelial cells, E2 increases mitochondrial proteins and decreases ROS production, an effect mediated by ER $\alpha$  but not  $\beta$  (74). Estrogens differentially regulate PGC-1 isoforms in **mouse** cerebral blood vessels: estrogen-regulated increase in mitochondrial biogenesis, function and protection against ROS seems to involve up-regulation of PGC-1 $\beta$  and down-regulation of PGC-1 $\alpha$  (30).

In the **mouse** liver, no differences between males and females were observed (71).

In **mouse** aorta, however, E2 decreases expression of respiratory chain and NRF genes thus inhibiting proliferation and migration of vascular smooth muscle cells (75). The discrepancy between down-regulation of nuclear-encoded genes by estrogen in the aorta vs. up-regulation in other tissues reflects the tissue-specificity of estrogen effects.

Much less is known of the effects of male hormones on mitochondrial structure and function. Testosterone regulates myocardial cell growth and the activity of some inner mitochondrial membrane proteins in **rodent** ventricular myocardium (76). In skeletal muscle L6E9 cell line, testosterone has no effect on markers of mitochondrial biogenesis, dynamics, and function (72). In 3T3-L1 adipocytes, while E2 induces mitochondrial proliferation and differentiation, testosterone shows opposite effects (44).

Thus, transcriptional pathways of mitochondrial biogenesis exhibit an important sexual and tissue specificity that could play an essential role in the pathogenesis of chronic diseases.

Sex-specific composition and function of mitochondria have been described in various tissues including liver, **adipocytes**, neurons, brain, skeletal muscle and heart at different degrees. The sex-specificity of mitochondria involves protein content and specific activity, phospholipid content of mitochondrial membranes, oxidant and anti-oxidant capacities, oxidative phosphorylation, and calcium retention capacities.

Other mitochondrial functions or mitochondrial properties may also differ between males and females but have not been investigated so far. More studies are thus needed to decipher the sex specificity of mitochondria.

Understanding the crosstalk between mitochondria and sex hormones may provide insights into the pathologies, mainly those associated with age (25). **The impact of gender differences in aging and pathologies will be described below.**

### MITOCHONDRIA ARE INVOLVED IN SEX-SPECIFICITY OF AGING AND PATHOLOGIES

Mitochondrial disorders are causally affected by mutations in either nuclear or mitochondrial genes involved in the synthesis of respiratory chain subunits or in their post-translational control. This can be due to mutations of the mitochondrial DNA which are transmitted by the mother, or mutations in the nuclear DNA. As they can affect all tissues, they are responsible for a large panel of pathologies including neuromuscular disorders, encephalopathies, metabolic disorders, cardiomyopathies, neuropathies, renal dysfunction etc... Because natural selection on mitochondria operates only in females, mutations may have had more deleterious effects in males than in females (35, 77). These mitochondrial diseases will not be discussed here.

Mitochondrial dysfunction is also a pivotal pathophysiological mechanism for many acquired chronic diseases like metabolic and cardiovascular disorders, and neurodegenerative diseases. Both sex- and gender-specific factors and risks affect the incidence progression and therapeutics of pathologies. Among these, biological factors are important and deserve a better attention.

While some sexual dimorphism has been described in healthy conditions in many tissues (see above), the sex-specific behavior of mitochondria is exemplified in pathologies (Table 2). Here we present aging and some pathologies with a sex-specificity in which mitochondria can play an important role.

#### a. Aging and longevity

Biological differences between the sexes, including differences in genetic and physiological factors may account for at least a part of the female advantage in human life expectancy (1). Mitochondria are thought to play a key role in aging and age-related diseases. The mitochondrial theory of aging links aging, exercise and diet. In many species including humans, females live longer than males, and are protected against cardiovascular diseases (6, 60). The positive effect of estrogens on anti-oxidant defenses and mitochondrial capacity may explain in part this difference. There are evidences for sex differences in the regulation of the cellular redox system (57). Mitochondria are an important source of free radical species and the most direct target for their damaging effects. Because they produce less ROS and have higher capacity of antioxidant defenses, mitochondria from females have higher resistance to ischemia/reperfusion (57). Posttranslational modifications of mitochondrial proteins can also modify ROS handling and play an important role in female cardioprotection (61). Estrogens promote mitochondrial biogenesis, efficiency and protection against oxidative stress (25). Mitochondria of females accumulate less calcium (65) and have a lower probability of PTP opening, thus protecting female cells against mitochondria-triggered cell death. These effects could be for a part mediated by estrogens. This argues in favor of a lower susceptibility to cell death of female cells.

Aging is characterized by decreased production of sexual steroid hormones especially in women after menopause. Decreasing levels of hormones promote tissue degeneration and age-related pathologies. Decline in sex steroid hormones and accumulation of mitochondrial damage may create a positive feedback loop that contributes to the progressive degeneration in tissue function during aging (25). In old but not senescent male and female monkeys, cardiac aging is characterized by decreased expression of glycolytic enzymes and reduced capacity for oxygen consumption in males but not females (78). This may explain why aging females live longer than males and are protected from cardiovascular diseases

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(78). In 24 month-old rats, cardiac mitochondrial function exhibits sexual dimorphism in the age-associated defects. In particular, old females exhibit a significant loss of mitochondrial function and increased relative H<sub>2</sub>O<sub>2</sub> production compared with their male counterparts (79), highlighting the protective effects of estrogens in younger females.

ROS have 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 damages in the aging cells. Targeting catalase (a detoxifying enzyme) to mitochondria increases murine life span and protects against age-related diseases (80). These intriguing results support the idea that mitochondrial ROS may be an important limiting factor in mammalian longevity (80). Study on skeletal muscle oxidative stress during aging shows that men may be potentially more vulnerable to oxidative damage than women (81). Conversely females produce less mitochondrial free radicals and have a higher content of antioxidant enzymes than males (50, 60, 61, 82). The effectiveness of estrogens against age-related diseases may arise in part from hormonal effects on mitochondrial function. Estrogen receptors and their signalling cascade have been implicated (60) in the control of antioxidant enzyme expression.

It thus appears that estrogens, mitochondrial capacity and reactive oxygen species are good candidates to explain the mechanism mediating delayed aging and cardiovascular risk in females.

### b. Cardiovascular diseases

Heart failure (HF) is a leading cause of cardiovascular mortality and morbidity in the Western world. It affects men at younger age than women (83). Premenopausal women experience less cardiovascular disease (CVD) compared with age-matched men. However, the rate of CVD development and mortality from cardiac disease exceeded that of men after menopause (6).

Metabolic disturbances, mitochondrial dysfunction and altered biogenesis are hallmarks of the failing heart (11, 84). In search for sex-differences in the development of heart failure, 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 (67). These differences could contribute to the protection of females against heart failure. On the opposite, E2 deficiency induces mitochondrial compromise through PGC-1 $\alpha$  downregulation, leading to the menopause-associated left ventricular concentric remodeling in female mice (85).

Sex-related specificity has also been found in heart failure induced by toxic agents like anthracyclines. Anticancer therapies by anthracyclines are limited by their cardiotoxicity. Anthracycline cardiotoxicity is considered as a complex multifactorial process involving oxidative stress and mitochondrial damages (86, 87). Female rats appear much less sensitive to the cardiotoxic effects of doxorubicin (the main anthracycline) than male rats (51, 88, 89). Energy metabolism, AMPK pathway, cardiolipins and mitochondria are involved in the protective effects against anthracyclines cardiotoxicity in females (51, 88). Likewise, adult tumor-bearing male spontaneously hypertensive rats (SHR) were shown to be more cardiosensitive to doxorubicin than females, an effect that is attributed to the greater activation of oxidative stress and apoptosis in male SHRs (89) without differences in tumor volume regression (89). The cardiorenal syndrome (CRS) that consists in metabolic, cardiac, and renal abnormalities is closely related to mitochondrial dysfunction and impaired mitochondrial biogenesis. Sex differences in the CRS have mainly been attributed to estrogen signaling in the liver, adipose tissue, skeletal muscles, and vascular tissues (90).

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Ischemic heart disease is the leading cause of morbidity and mortality in both men and women but women have lower risk before menopause (91, 92). Ischemia and post-ischemic 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 (23). Female **rat** hearts are more resistant to oxygen deprivation (93, 94), and ischemic reperfusion injury induces lower infarct size in female than male rats (95). Similar observation was made in patients with acute myocardial infarction. Primary percutaneous coronary intervention results in better myocardial salvage in women than men (96). Lower calcium uptake rate and maintained membrane potential under high calcium in female cardiac mitochondria (65) may contribute to protection of female cardiomyocytes from ischemia/reperfusion injury. Properties of cardiac mitochondria from castrated female **rats** are similar to those observed in mitochondria from control females after ischemia-reperfusion (97), suggesting a role for estrogens in protection against cell injury.

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 (98). Vascular diseases are instrumental in aging, as well as cardiac and neurological disorders. Blood vessels are the place of significant sexual dimorphism. Estrogen plays an essential role in the regulation of vascular tone and in the pathophysiology of cardiovascular diseases. Endothelial ER $\alpha$  **is involved** in flow-mediated remodeling of resistance arteries *in vivo* (99). The beneficial effects of estrogens on the vasculature result from their antioxidant properties and their ability to enhance relaxation by modulating synthesis of endothelium-derived vasodilators (100). ER $\alpha$  and  $\beta$  are both expressed in vascular smooth muscle and endothelial cells. However, they can affect differently the expression of mitochondrial proteins depending on the cell type and the vascular bed. In **rat** cerebral blood vessels, estrogen increases mitochondrial efficiency and reduces oxidative stress (101), while in mouse aorta, E2/ER $\beta$  pathway mediates the down-regulation of mRNAs for nuclear-encoded subunits in each of the major complexes of the mitochondrial respiratory chain (75). This may be due to the ER $\alpha$ /ER $\beta$  balance in different cell types (75). Other sex differences may be revealed in response to cell stress. Exposition of **rat** smooth muscle cells to ultraviolet radiation induces an upregulation of survival proteins in cells from females, while in cells from males pro-apoptotic proteins and loss of mitochondrial membrane potential are increased (102). 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 (103).

Thus clearly, part of the protective effect of female sex in cardiovascular diseases is linked to better protection of mitochondrial function and content in female heart and vessels and better ability to handle calcium and to decrease ROS production.

### c. Metabolic diseases

The prevalence of metabolic diseases like obesity or type 2 diabetes is steeply increasing. Although gender based factors like nutritional state, way of life and environment undoubtedly play significant role, clinical and experimental data indicate that sex-differences, mainly driven by sex hormones, induce different presentations in young versus elderly population.

Sex differences in metabolism have been observed for a long time. Males and females differentially rely on fuels for energy consumption, males being more reliant on carbohydrates and females on lipids. Estrogen signaling pathways are involved in hepatic

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and cardiac lipid metabolism and in the counter regulatory response to hypoglycemia (104). In the body, estrogens protect against adiposity, insulin resistance, and type II diabetes, and regulate energy intake and expenditure (see (105) for review).

Available data suggest that the pathophysiology of the metabolic syndrome and its contribution to the relative risk of cardiovascular events and heart failure show gender differences (106). Glucose tolerance progressively declines with age, and there is a high prevalence of type 2 diabetes (T2D) in the elderly people with increased incidence in men. In rodent models, T2D occurs more frequently or even exclusively in males (107, 108). Clinical trials and animal studies have revealed that loss of circulating estrogens induces rapid changes in whole body metabolism, fat distribution, and insulin action (reviewed in (105)). Recent investigations suggest that E2/ER $\alpha$  elicits the metabolic effects of estrogens by genomic, non-genomic and mitochondrial mechanisms that regulate insulin signaling, substrate oxidation, and energetics (105). Indeed, estrogen deficiency as occurs at menopause contributes to the elevated risks of diabetes in women (105, 108). Male hormones also play a role. Subjects with low testosterone levels have a threefold higher prevalence of the metabolic syndrome than their normal counterparts. Low testosterone levels are associated with lower oxidative profile, showing that low testosterone levels and impaired mitochondrial function promote insulin resistance in men (109).

There is increasing evidence of gender-related differences in risk factors, clinical manifestation and sequelae of obesity and diabetes. In diabetic patients, the incidence of cardiovascular diseases is high, with a higher incidence in women than in men (106). **In rats, female cardiac mitochondrial** respiration seems to be more affected by streptozotocin-induced diabetes **than the male one** but insulin treatment restores mitochondrial function more effectively in female heart (110). Diabetes and insulin treatments differentially affect cerebral mitochondrial energy metabolism in the male and female rats (111), diabetes decreasing mitochondrial respiration exclusively in males.

A sexual dimorphism in the age-associated impairment of pancreatic beta cell function in elderly rats has been demonstrated with females retaining a higher glucose-stimulated insulin secretion. The potential mechanism could be related to the sexual differences in mitochondrial biogenesis and function (112).

A sex-specificity has also been observed in response to high fat diet. High fat feeding induces insulin resistance and metabolic syndrome that may progress to T2D. In brown adipose tissue, high fat feeding induces enhanced mitochondrial differentiation in male rats, while females exhibit a decrease in mitochondrial functionality but a rise in mitochondrial proliferation (113). In liver, high fat feeding, induces higher levels of mitochondrial protein and enhanced oxidative capacity per mitochondrion in female compared to males (114). **Thus there is a sex-specific response to excess nutrients which involves mitochondria.**

Calorie restriction is one of the most effective ways to increase lifespan. Again a sexual dimorphism is observed in the response to chronic calorie restriction. Hearts of female mice retain a more dynamic triacylglyceride pool than males, while males respond with greater metabolic remodeling of cardiac lipid dynamics (115). During starvation, neurons from males more readily undergo autophagy and die, whereas neurons from females mobilize fatty acids, accumulate triglycerides, form lipid droplets, and survive longer (116). Adult women exposed to prenatal nutrient restrictions exhibit increased risk factors of cardiovascular disease, while men exposed to the same condition do not (35).

Thus, whole body metabolic state and metabolic diseases present with sexual dimorphism involving for part the interplay between steroid hormones and mitochondria.

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### d. Neurodegenerative diseases

There are important sex differences in the brain that seem to arise from biological as well as psychosocial factors (7). Many neurodegenerative disorders exhibit a gender-dependency with a higher incidence in men than women. Most of the neurodegenerative disorders involve either causally or consequently a dysfunction of mitochondria. In general, females are protected from neurological disorders at young ages suggesting an important role for sexual hormones. Males would be more susceptible to central nervous system injury due to the absence of the protective role of female hormones, by processes involving mechanisms converging on mitochondria (reviewed in (12)). Increased resistance of females to brain injury may be related to a lower mitochondrial oxidative stress and higher antioxidant defenses.

Alzheimer's disease (AD) is by far the most common neurodegenerative dementia among the elderly. It represents 60% of all dementia cases (117). The incidence of AD is lower in young women than in men but is increased among postmenopausal women (117, 118). These observations suggest that estrogens have important protective effects against the development of AD. Mitochondrial dysfunction is an underlying event in AD progression including decreased bioenergetics, increased ROS production and decreased mitochondrial biogenesis (12, 118-122). AD is characterized by the accumulation of amyloid- $\beta$  ( $A\beta$ ) plaques in the brain. In the presence of  $A\beta$ , mitochondria produce free radicals that trigger both the mitochondrial and the extra-mitochondrial pathways of apoptosis. Mitochondria from young female rats are protected against  $A\beta$  toxicity, generate less reactive oxygen species, and release less apoptogenic signals than those from males. However, all these advantages are lost in mitochondria from old females (123). Furthermore, the capacity of estrogens to delay the loss of endothelial vasodilatation function and to protect vascular mitochondria may significantly affect the occurrence and course of a number of age-related diseases like Alzheimer disease, stroke, and vascular dementia (30). Estrogens can play a protective role in the maintenance and function of mitochondria, which are vulnerable organelles in AD (122, 123). Among others, beneficial effects of estrogen include potentiated mitochondrial bioenergetics, and promotion of antioxidant defense against free radicals (31). Interestingly, estradiol protects against  $A\beta$  neurotoxicity by limiting mitochondrial dysfunction via activation of antiapoptotic mechanisms (124). However, estrogens may be deleterious in AD when applied late after the initiation of the pathology or on already compromised mitochondria or too late after menopause (31). Finally, neuroprotection by sex steroid hormones also include progesterone and testosterone (121, 125). Thus, the higher prevalence of AD in older women involves the drop of sex hormones and its deleterious effect on mitochondrial bioenergetics and oxidative stress. Therefore new therapeutic strategies should take into account the sex and gender aspects of AD.

Gender is one of the risk factors in amyotrophic lateral sclerosis (126). Amyotrophic lateral sclerosis (ALS) is the most frequent motor neuron disorder in adults. ALS is a devastating neurodegenerative disorder that is more prevalent in males than in females. Men are almost twice as likely to be affected as women, but again the difference is less significant when the disease onset is at an older age (127, 128), suggesting that declining estrogen levels may result in loss of neuroprotection. Female G93A mutant SOD1 transgenic mice, a model mimicking ALS, have a slower disease time-course than males, consistent with the gender difference observed in humans (129). The protective role of E2 is evidenced by the fact that in these mice, ovariectomy accelerates the progression of the disease, an effect that can be



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reversed by chronic estrogen treatment (129). Early and gender-specific differences in spinal cord mitochondrial function and oxidative stress markers have been described in this model (130). Brain and spinal cord mitochondrial  $\text{Ca}^{2+}$  capacity decreases before disease onset (131), suggesting that abnormal  $\text{Ca}^{2+}$  homeostasis is a primary pathogenic event. Estrogen-mediated neuroprotection in experimental ALS involves decreased mitochondrial  $\text{Ca}^{2+}$  overload and damage in females (63). The neuroprotective effects of estrogen could be in part mediated through decreased mitochondrial  $\text{Ca}^{2+}$  accumulation and dependent on the mitochondrial estrogen receptor and cyclophilin D (63). Furthermore, women with ALS often have a history of earlier menopause than controls. Taken together, these epidemiological observations imply estrogen as a protective factor against ALS. This protection has to be prolonged and start early in life, since studies in postmenopausal women did not show neuroprotective effects of estrogen replacement on incidence or progression (132).

Parkinson disease also occurs more frequently in men than in women (133, 134). Accumulating evidence suggests that mitochondrial dysfunction due to an impairment of mitochondrial energy production and subsequent oxidative stress may be involved (133, 135). Oxidative stress is more pronounced in male than in female mouse mesencephalic neurons (135). Remarkably, a set of genes associated to mitochondrial function appears to be deregulated mainly in male Parkinson Disease patients (136). An important role of the regulator of mitochondrial biogenesis PGC-1 $\alpha$  was identified as critical for gender-dependent vulnerability to Parkinson's disease (137) and as a potential therapeutic target (136).

Many neurodegenerative diseases like AD, ALS and Parkinson diseases are associated with endoplasmic reticulum (ER) stress, stimulation of the unfolded protein response (UPR) and autophagy. Autophagy plays an essential role in the clearance of aggregated toxic proteins and degradation of the damaged organelles like mitochondria. It remains to be established if and how these pathophysiological processes exhibit sex differences that could contribute to the observed gender differences of these pathologies.

It is increasingly acknowledged that a number of neurodegenerative diseases involve mitochondrial dysfunction that induces decreased ATP production, altered calcium handling and increased ROS production. These neurodegenerative diseases show higher prevalence in men compared to women before menopause. Recent data point to a role for mitochondria in the sexual dimorphism of neurodegenerative diseases having potential implications for the development of innovative neuroprotective therapy.

## CONCLUSION

Mitochondria are pivotal organelles for cell fate. Their involvement in chronic disorders is increasingly acknowledged. It clearly appears that mitochondria are also the place of marked sex-specificities. They are involved in the protective effects of female sex such as neuroprotection against central nervous system injury, Parkinson disease, cardioprotection of the infarcted heart, protection against heart failure or idiopathic cardiomyopathies, longevity and oxidative stress (Table 2). Finally, mitochondria are also involved in the sex differences in the toxicity of xenobiotics and drugs.

The positive effect of estrogens on anti-oxidant defenses and mitochondrial capacity may explain in part why women live longer than men and have less age-related diseases at least before menopause (Figure 4). Decreasing levels of hormones promote tissue degeneration and age-related pathologies. Findings suggest that mitochondria participate in the lower susceptibility to cell death of female cells. The mitochondrial theory of aging stipulates that



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mitochondrial dysfunction causes increased ROS production inducing cumulative damage to the mitochondria.

Mitochondria are key players in the regulation of glucose and lipid utilization and are clue targets in metabolic pathologies like diabetes and obesity.

Alterations of mitochondrial mass and function play an important role in cardiovascular diseases. Mitochondrial mass is decreased in heart failure, resulting in an energetic imbalance between the increased demand to the myocardium and the lower capacity to produce energy. Females are less susceptible to heart failure and this may involve mitochondrial biogenesis.

Dysregulation of calcium signaling in mitochondria has been implicated in several neurodegenerative diseases. Increased resistance of females to brain injury may also be related to a lower mitochondrial oxidative stress and higher antioxidant defenses. Whether triggered by environmental or genetic factors, mitochondrial dysfunction occurs early in the pathogenic process of Parkinson disease for example.

Sex differences in the occurrence, development and outcome of pathologies become more and more obvious. Yet, the consideration of sex differences in clinical and experimental research is still poorly acknowledged. Pathologies with a better prognosis in women involve protective effects of estrogens and mitochondria. Pathologies with a women disadvantage like autoimmune disorders seem to be less dependent on mitochondrial dysfunction as primary factor. The advantage of a better functioning of mitochondria in females involves positive action of estrogens but may also be driven by the evolutionary selection of mitochondria in female background. Further research should consider both sexes not only to better understand the pathophysiology of the diseases but also to prompt more appropriate therapeutic interventions. Taking into account sex specificity in aging and pathologies would allow developing more focused drugs and therapeutic strategies. Studies are thus necessary for delineating the consequences of mitochondrial sex-specificity in the pathophysiology of chronic diseases and to elaborate new therapeutic interventions.

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**Table 1. Sex-specificities of mitochondria in different organs**

TISSUE	Species	MITOCHONDRIAL MECHANISMS / PROPERTIES	SEX DIFFERENCE	REFS
<b>LIVER</b>	<b>Rodent</b>	ADP-stimulated respiration	F>M	(36-38)
		Cardiolipin content	F>M	(38)
		Protein content	F>M	(38)
		High fat diet induced protein content	F>M	(114)
		High fat diet induced OXPHOS capacities	F>M	(114)
		ROS production	F<M	(59, 60)
		mtDNA/nDNA	F>M	(16)
		Mitochondrial biogenesis factors	F=M	(71)
<b>CEREBRAL ARTERIES</b>	<b>Rodent</b>	Basal and maximal respiration	F>M	(39)
		ATP production	F>M	(39)
		Proton leak	F>M	(39)
		Spare respiratory capacity	F>M	(39)
<b>BRAIN</b>	<b>Rodent</b>	ROS production	F<M	(40)
		Oxidative damage	F<M	(58)
		Glutathione cycle (in young rats)	F<M	(41)
		NADH-linked respiration	F>M	(40)
		ETC transport, ATP production	F>M	(41)
		Functional capacities	F>M	(42)
		Mitochondrial biogenesis	F<M	(71)
		Fatty acid utilization	F>M	(12)
		Protein utilization	F<M	(12)
		Calcium uptake capacity	F<M	(63)
	<b>Human</b>	Calcium kinetics	F=M	(37)
		Enzyme activities (CS, SDH, MTT)	F>M	(43)
<b>WHITE ADIPOSE TISSUE</b>	<b>Rodent</b>	Functional mitochondria	F>M	(44)
	<b>Human</b>	Genes involved in mitochondrial functions	F>M	(49)
<b>BROWN ADIPOSE TISSUE</b>	<b>Rat</b>	Functional mitochondria	F>M	(46, 113)
<b>PANCREATIC BETA-CELLS</b>	<b>Rat</b>	Glucose-stimulated insulin secretion	F>M	(111)
<b>SKELETAL MUSCLE</b>	<b>Rat</b>	Functional mitochondria	F>M	(47)
		mtDNA and TFAM protein content	F>M	(48)
		OXPHOS activities	F>M	(48)
	<b>Human</b>	mtDNA/nDNA	F>M	(16)
		Intracellular lipid content	F>M	(53)
Protection against oxidative stress	F>M	(81)		
<b>HEART</b>	<b>Rodent</b>	Mitochondrial content	F<M	(50)

## Mitochondria and sex differences

Mitochondrial efficiency and differentiation	F>M	(48)
Oxygen consumption rate (baseline)	F=M	(51)
Cardiolipin content	F=M	(51)
Glutamate/malate stimulated respiration	F>M	(52)
Other substrates stimulated respiration	F=M	(52)
ADP/O ratio	F>M	(52)
Fatty acid utilization during exercise	F>M	(54)
ROS production	F<M	(50, 61)
Calcium uptake rate	F<M	(65)
Calcium retention capacity	F>M	(37)

ROS, reactive oxygen species; MTT, mitochondrial reductase; TFAM, mitochondrial transcription factor A; mtDNA, mitochondrial DNA; CS, citrate synthase; SDH, succinate dehydrogenase.

## Mitochondria and sex differences

**Table 2. Important role of mitochondria in sex-dependency of pathologies.**

AGING OR PATHOLOGY	PROPERTIES AND MITOCHONDRIAL INVOLVEMENT	SEX DIFFERENCES	REFS
<b>AGING</b>			
<b>Humans</b>	Life expectancy	F>M	(1)
	Cardiovascular diseases	F<M	(9, 60)
<b>Rodents</b>	Oxidative stress	F<M	(25, 57, 81)
	Mitochondrial calcium accumulation	F<M	(65)
	Susceptibility to cell death	F<M	(65)
<b>Old rats</b>	H <sub>2</sub> O <sub>2</sub> production	F>M	(79)
	Mitochondrial function	F<M	(79)
<b>Aging monkeys</b>	Expression of glycolytic enzymes	F>M	(78)
	Oxygen consumption	F>M	(78)
<b>CARDIOVASCULAR DISEASES</b>			
<b>Hypertrophy and heart failure</b>	Age of onset	F>M	(83)
	Mortality and rate of development	F<M	(83)
	Before menopause	F<M	(9)
	After menopause	F>M	(9)
	Mitochondrial biogenesis decrease	F<M	(67)
<b>Anthracycline toxicity</b>	Cardiotoxic effects	F<<M	(51, 88, 89)
	Cardiolipin content	F>M	(51, 88)
	Mitochondrial dysfunction	F<M	(51, 88)
	AMPK decrease	F<<M	(51, 88)
	Oxidative stress and apoptosis	F<M	(89)
<b>Cardiorenal syndrome</b>	Oxidative capacity decrease	F<M	(90)
<b>Ischemic heart disease</b>	Risk at young age	F<M	(91, 92)
	Resistance to hypoxia	F>M	(93, 94)
	Infarct size after MI (rats)	F<M	(95)
	Myocardial salvage after coronary intervention	F>M	(96)
	Reperfusion injury	F<M	(65)
	Resistance to cell death	F>M	(57)
<b>METABOLIC DISEASES</b>			
<b>Type II Diabetes</b>	Incidence in humans	F<M	(106)
	Induction in rodents	F<M	(107, 108)
	Mitochondrial respiration decrease (rat heart)	F>M	(111)
	Insulin efficiency	F>M	(111)
	Pancreatic beta cell function in aging	F>M	(112)
<b>High fat diet</b>	<i>In brown adipocytes:</i>		
	Mitochondrial differentiation	F<M	(113)
	Mitochondrial proliferation	F>M	(113)
	<i>In liver:</i>		
	Mitochondrial protein level	F>M	(114)
	Oxidative capacity	F>M	(114)
<b>Starvation</b>	<i>In neurons:</i>		
	Autophagy	F<M	(116)
	Cell death	F<M	(116)

## Mitochondria and sex differences

NEURODEGENERATIVE DISORDERS			
Alzheimer disease	Neurological disorders at young age	F<M	(12)
	Incidence in young humans	F<M	(117, 118)
	Protection against A $\beta$ toxicity	F>M	(123)
	Mitochondrial ROS production in neurons	F<M	(12, 118, 119, 121)
Amyotrophic lateral sclerosis	Prevalence at young age	F<M	(127, 128)
	Disease course	F<M	(129)
	Mitochondrial Ca <sup>2+</sup> accumulation	F<M	(63)
Parkinson disease	Mitochondrial function	F>M	(130)
	Occurrence	F<M	(126)
	Mitochondrial dysfunction	F<M	(133)
	Oxidative stress	F<M	(135)
	Mitochondrial function genes	F>M	(136)

### FIGURE LEGENDS

**Figure 1. Mitochondria are at the heart of neurological, metabolic and cardiovascular disorders as well as aging.** Sexual dimorphism of these pathologies involves the sex-specificity of mitochondrial functions in a tissue specific manner.

**Figure 2. Mitochondria life cycle.** Mitochondria life cycle involves mitochondrial biogenesis, mitochondrial dynamics and quality control involving mitophagy. Mitochondrial biogenesis is initiated by an energetic disequilibrium that is sensed by two signaling pathways: the AMP-dependent protein kinase (AMPK) and the deacetylase sirtuin 1. Increased expression or activity of the master regulators of mitochondrial biogenesis PGC-1 $\alpha$  et  $\beta$  activates the expression of the nuclear respiratory factors 1 and 2 (NRFs) which induces the expression of the mitochondrial transcription factor A (TFAM) which translocates to mitochondria, binds to mtDNA and activates its transcription and replication. NRFs together with other transcription factors (ERRs PPARs...) activate the expression of nuclear-encoded mitochondrial proteins (mt proteins) which are imported to mitochondria. Mitochondria then undergo fusion and fission cycles known as mitochondrial dynamics. Defective mitochondria (red) can be eliminated in the autophagy vesicles through a process called mitophagy.

**Figure 3. Influence of estrogens on mitochondrial functions in most of the tissues.** The binding of estrogens (E2) to their cytosolic receptors induces the translocation of the complex estrogen/estrogen receptors (ER) to the nucleus. The interaction of this complex with nuclear DNA results in the transcription of *Pgc-1 $\alpha$*  and *Nrfs* which stimulate mitochondrial biogenesis and antioxidant defenses. Estrogens also interact with estrogen receptors bound to mitochondrial DNA (mtDNA), thereby leading to transcription and replication of the mtDNA. Mitochondrial biogenesis favors fatty acid utilization, oxidative capacity and ATP production. Estrogens can also bind to G-protein-coupled estrogen receptors (GPER) at the plasma membrane, activating NF- $\kappa$ B in a MAPK-dependent manner. This results in the transcription of genes encoding antioxidant enzymes, reinforcing in particular the antioxidant defenses of the mitochondria. Therefore, the mitochondria undergo less ROS-induced damages and they consequently display higher fatty acid utilization, oxidative capacity and ATP production. Inasmuch as the antioxidant systems preserve the integrity of the mitochondria, the release of the pro-apoptotic factors by these organelles is reduced, ensuring decreased cell death and preservation of organ functions. The higher calcium retention capacities generally observed in female mitochondria also contributes to the low apoptotic factor release.

**Figure 4. Sex-dependent pathophysiological mechanisms involving mitochondria.** Aging and chronic diseases with a mitochondrial component exhibit gender differences with lower incidence in women before menopause. The pathophysiological mechanisms involve decreased mitochondrial function, altered calcium handling and increased ROS generation that lead to PTP opening and cell death mainly in males while mitochondrial function, oxidative stress and calcium handling are better preserved in females. Estrogens seem to play a major role but selection of X chromosome and mitochondrial genome could also be involved.

# Cardiovascular diseases

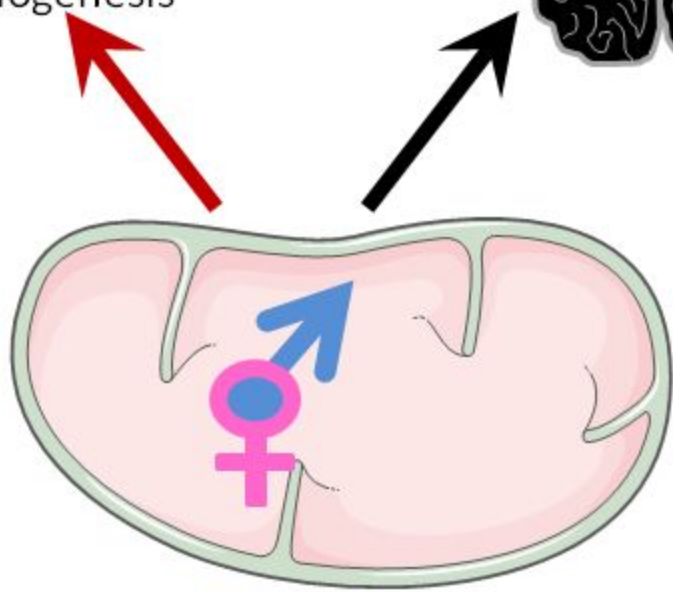


Oxidative stress  
Calcium handling  
Mitochondrial biogenesis

# Neurological disorders



Oxidative stress  
Calcium handling  
Mitochondrial efficiency  
Mitochondrial biogenesis



# Metabolic diseases



Oxidative stress  
Substrate use

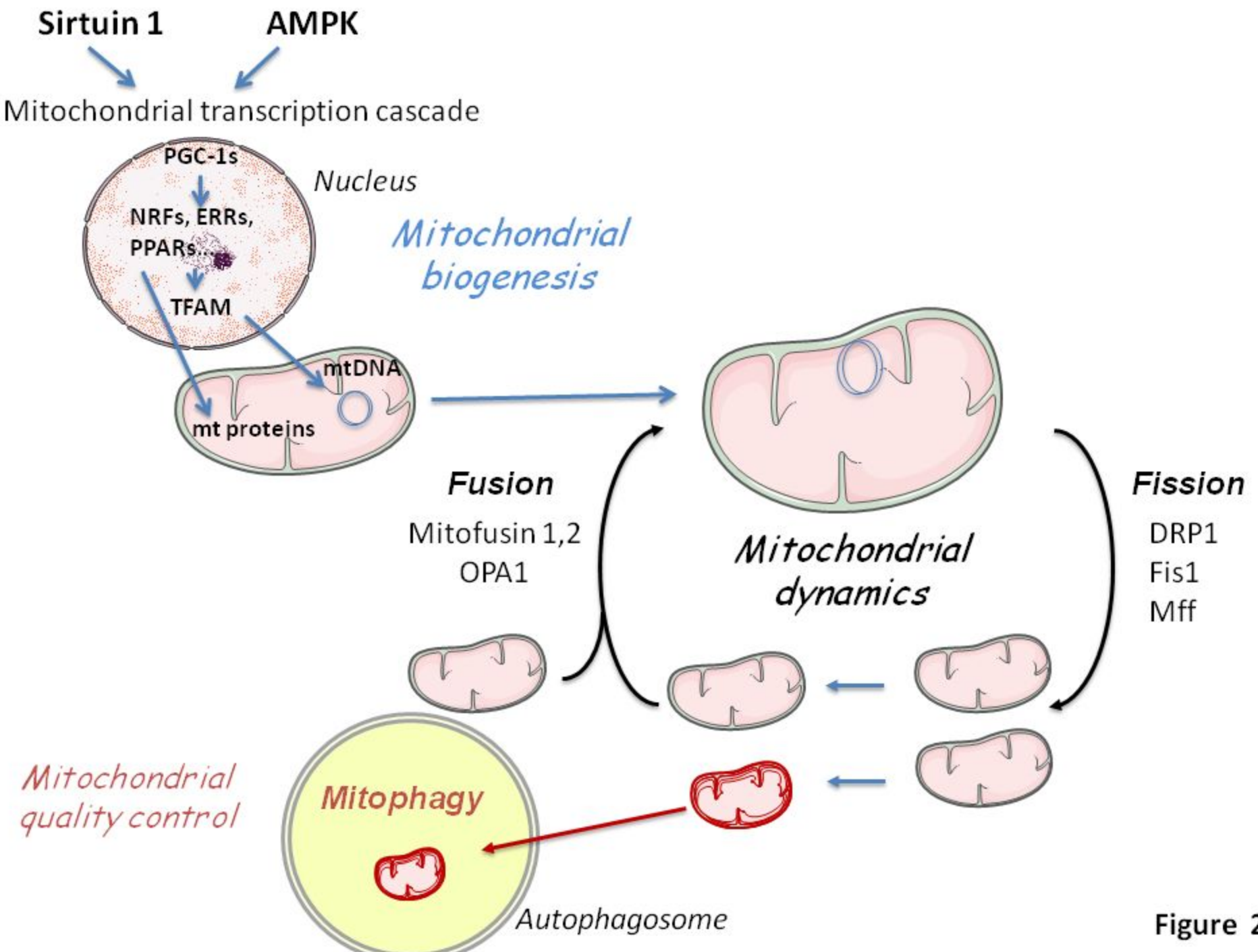
# Aging



Oxidative stress  
Calcium handling  
Mitochondrial efficiency  
Mitochondrial biogenesis

Figure 1





**Figure 2**

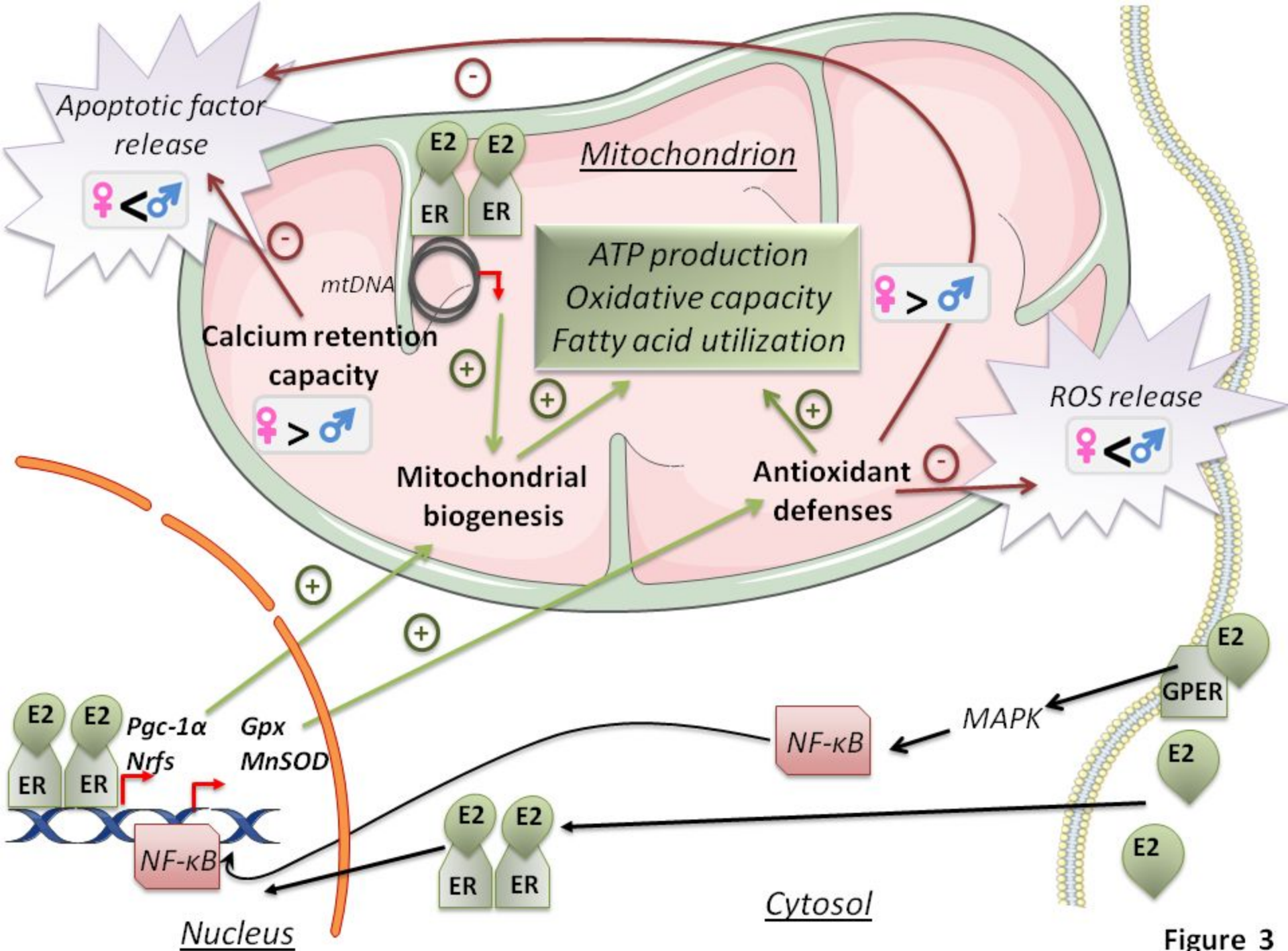
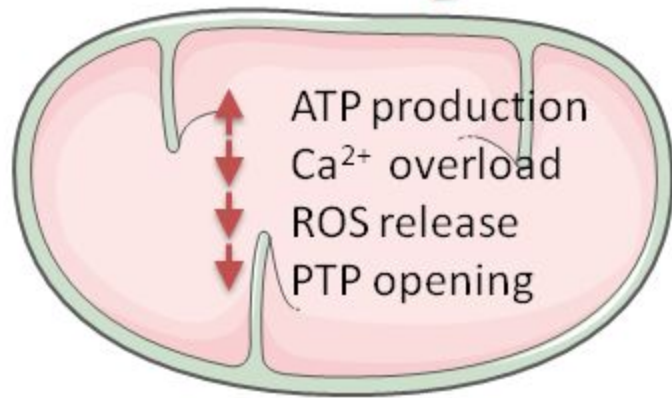


Figure 3

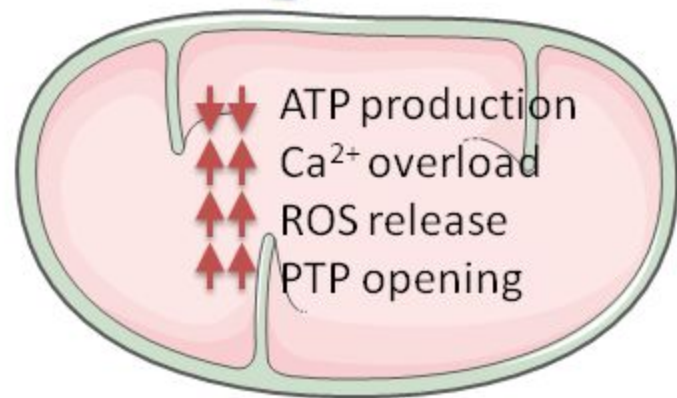
Aging,  
Cardiovascular diseases  
Metabolic disorders  
Neurodegenerative disorders,

**Estrogens** ⊖  
*X chromosome*  
*Mitochondrial genome*



Preserved mitochondria  
Oxidative balance  
PTP closing

**Cell survival**



Damaged mitochondria  
Oxidative stress  
PTP opening

**Cell death**

Figure 4