Chapter 14
Sex-Related Pathophysiological Differences in Cardiac Mitochondria: Role of Estrogens

Natalia Pavón, Francisco Correa-Segura, Elizabeth Lira-Silva, and Alfredo Cabrera-Orefice

Abstract A higher prevalence of cardiovascular diseases has been observed for long time in men; although initially associated with stressful working situations that women supposedly did not deal with. However, the subsequent inclusion of women in all kind of formal jobs proved this idea wrong since cardiac affections still occur more frequently in men. Apart from clinical studies, considerable research has been performed in order to unveil the pathophysiological causes accounting for this sex-related difference. Nowadays, it is known that female hormones, particularly estrogens, have cardioprotective effects; thus pointing them out as key factors in the development of cardiovascular disorders. Estrogens regulate the heart function not only at the organ/cellular levels but also at the mitochondrial level. The effects of estrogens on mitochondria may result from their binding to nuclear and mitochondrial estrogen receptors, which modulate gene expression and signaling pathways or directly by interacting with proteins and modifying physicochemical properties of mitochondrial membranes. In female animal models, estrogens deprivation leads to a number of heart mitochondrial impairments and higher sensitivity to ischemia/reperfusion. Despite we have gained substantial descriptive evidence of these effects in heart, the molecular basis remains unclear. This book chapter aimed to revisit the current knowledge about sex-related differences in cardiac pathophysiology, emphasizing on the role of estrogens in heart mitochondrial function under normal conditions and after ischemia/reperfusion injury.

Keywords Mitochondria · Estrogens · Oophorectomy · Heart · Cardiovascular disease · Gender difference · Ischemia/reperfusion

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N. Pavón (✉) · E. Lira-Silva
Departamento de Farmacología, Instituto Nacional de Cardiología, Ciudad de México, Mexico
e-mail: pavitonat@yahoo.com.mx

F. Correa-Segura
Departamento de Biomedicina Cardiovascular, Instituto Nacional de Cardiología, Ciudad de México, Mexico

A. Cabrera-Orefice
Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands

Introduction: "A Females' Thing"

Until the World War I, it was common to believe that heart stroke was a "men's illness" due to the low prevalence of this condition in women. In those times, cardiovascular affections were mostly related to high stress situations that women, typically staying home, apparently did not deal with. In the course of the mid-twentieth century, the majority of men continued working in the stressful fields, factories and offices; whereas women were still far from such a pressure. Nowadays, in spite of the presence of women in all working fields, their susceptibility to suffer a heart attack is still lower. Thus, a sex-related explanation for this condition becomes essential.

Testosterone and other androgenic steroids have been associated with myocardial ischemia, sudden cardiac death and hypertension in males, particularly athletes, leading to the view that these hormones are detrimental for the cardiovascular system [1–3]. In contrast, different intervention studies rather suggest a beneficial effect of natural circulating androgens on coronary heart disease in males [4]. Indeed, there is substantial evidence that physiological testosterone levels have favorable effects on blood vessels and cardiovascular system due to the activity of aromatase, which catalyzes the interconversion of testosterone to estradiol. Inhibition of this enzyme increases the fatty streak lesions in male mice at the same extent as castration does [5]. It has also been demonstrated that the aromatizable androgen dehydroepiandrosterone (DHEA) prevents atherosclerosis in intact cholesterol-fed rabbits, whereas non-aromatizable androgens have no effects [6]. Cardiovascular diseases have been described to increase in women entering menopause [7]. This female stage is characterized by a decline in hormone production by the ovaries, mainly estrogens [8]. It is also known that cardiovascular diseases show a better prognosis in pre-menopausal women than in men. Several epidemiological studies have also shown that the risk of atherosclerotic disease is low in pre-menopausal women and increases dramatically after menopause. Reasonably, estrogens have been pointed out as the causative factors of this sex-related cardiac difference.

However, current knowledge about testosterone/androgens effects on cardiovascular system remains controversial; therefore, further studies are required to unveil the pathophysiological mechanisms exerted by these hormones, especially in humans.

Estrogens and Heart

Estrogens are considered traditionally as female sex hormones; most likely due to their high concentrations and the remarkable physical changes they promote in females. Nevertheless, these steroid hormones are also synthesized in males and participate in a number of physiological and reproductive processes; e.g. spermatogenesis and erectile function [9]. There are three main estrogens with hormonal activity: 17β-estradiol, estrone and estriol [10]. Above all, the most potent estrogen

in circulation is 17β-estradiol. In general, estrogen hormones not only regulate reproductive system functions, but also participate in other physiological processes such as cognition [11], cardiovascular function [12], immunity [13] and mineral and bone metabolism [14].

The pleiotropic role of estrogens arises essentially from their binding to intracellular estrogen receptors (ERs). These hormone-receptor complexes are translocated into the nucleus to regulate gene expression [15]. Two isoforms of ERs have been identified: estrogen receptor α (ERα) and β (ERβ). These receptors are encoded respectively by the *ESR1* and *ESR2* genes in humans. Both isoforms belong to the superfamily of nuclear receptor transcription factors [16]. Estrogen hormones bind similarly to the two isoforms since the DNA- and ligand-binding domains are highly conserved [17]. In this regard, it has been shown that ERs may trigger signaling via both genomic and non-genomic pathways [18]. Recent studies have also evidenced that a G protein-coupled receptor with a high affinity for estrogens, known as GPER or GPR30, is present in heart and may modulate rapid signaling events [19–21], even in the presence of the so-called weak estrogens; i.e. estrone and estriol.

The presence of functional ERs has been demonstrated in cardiac myocytes and fibroblasts from both male and female rats [22]. Interestingly, the group of Regitz-Zagrosek has shown that ERα is localized in the intercalated discs, adjacent to the sarcolemma and in some but not all nuclei; besides, it exhibits a striated pattern with troponin T [23]. In rats with dilated cardiomyopathy, ERs are up regulated in both genders; the expression of ERs is remarkably higher in females though. Furthermore, the intercalated discs, which are critical sites for the normal propagation of electrical stimuli in cardiac myocytes, undergo significant changes in injured hearts. The intercalated discs are composed by gap junctions, desmosomes and adherens junctions. The gap junctions regulate electrical coupling, while desmosomes and adherens junctions integrate intermediate filaments and anchor myofibrils to the sarcolemma. Taken together, these data are particularly relevant and suggest that estrogens and their receptors could modulate not only the function, but also maintain normal cell–cell interactions between myocytes.

Role of Estrogens in Heart Mitochondria

A new perspective of estrogenic regulation emerged after the discovery of estrogen receptors α and β within mitochondria [24, 25]. This finding suggests that estrogens may modify directly the mitochondrial function and energy metabolism, although depending on the cell type. Prior to describe those effects, we have included a brief description of mitochondrial energy metabolism in cardiac cells for a basic biochemical understanding.

In aerobic eukaryotes, mitochondria are the major source of adenosine triphosphate (ATP), which is synthesized in the oxidative phosphorylation (OXPHOS) pathway. Four membrane oxidoreductases transfer electrons from different reduced electron carriers to the oxygen (O_2). These multi-subunit complexes (I, II, III and

IV) constitute the mitochondrial respiratory chain; a.k.a. electron transport chain. Complexes I, III and IV use the energy released during the electron transfer to translocate protons from the mitochondrial matrix to the intermembrane space; hence generating an electrochemical gradient of protons that is used by the F_1F_O-ATP synthase (complex V) to drive the ATP synthesis [26]. The establishment of the mitochondrial transmembrane potential ($\Delta\Psi_m$) by the respiratory chain is also important for other processes, such as metabolite transport, protein import, calcium (Ca^{2+}) uptake and fusion/fission [27, 28].

In heart, the excitation–contraction coupling and optimal myocardial performance has to be supported by the OXPHOS system, which keeps providing ATP in both systolic and diastolic periods. During maximal demand, cardiac muscle uses up to 90% of its oxidative capacity; i.e. cardiac cell function relies mainly on mitochondrial energy metabolism [29]. Finely tuning of ATP synthesis [30] is achieved when all OXPHOS components work tightly coupled; otherwise ATP yield is compromised leading to energy deficits and cardiac impairment [31]. Even though cardiac mitochondria are essentially dedicated for aerobic energy metabolism, these organelles are also involved in steroidogenesis, ion homeostasis and biosynthesis of heme groups, lipids, aminoacids and nucleotides [32–34].

The presence of ERs allows regulation of gene expression by estrogens not only in the nuclear genome but also in the mitochondrial genome [35]. It should be noted that the majority of mitochondrial proteins are encoded in the nuclear DNA (nDNA), synthesized in the cytosol and later imported into mitochondria. Nonetheless, few subunits of the OXPHOS complexes, as well as tRNAs and rRNAs are encoded in the mitochondrial DNA (mtDNA) and synthesized in the matrix [36]. In particular, estrogens regulate the mitochondrial metabolism, biogenesis and apoptosis by modulating the expression of a number of nDNA- and mtDNA-encoded proteins; e.g. subunit II of cytochrome *c* oxidase, PGC1α, Bax and Bcl-2 [37–41].

On the other hand, estrogen effects on mitochondrial functions are not only limited to gene regulation. For example, in the brain, estrogens may bind directly with complex V inhibiting ATP synthesis; whereas in heart, interaction of estrogens with this enzyme increases its activity suggesting a direct role in mitochondrial ATP production [42]. Importantly, estrogens have other effects that do not involve interactions with proteins or genes; instead they can directly modify the biophysical properties of mitochondrial membranes, such as microviscosity [43].

Sex-Related Differences in Cardiac Sensitivity to Ischemia/reperfusion Damage

Ischemia/Reperfusion (I/R) in Heart

Myocardial infarction, stroke and peripheral vascular disease occur when myocardial tissue receives insufficient blood supply; i.e. ischemia. The lack of oxygen and

nutrients results in lower mitochondrial ATP production, increased lactate production, cytosolic pH acidification, Ca^{2+} overload and multiple ion and redox imbalances [44]. The level of cardiac tissue damage depends directly on the extent of blood supply reduction and the length of the ischemic interval [45]. Paradoxically, restoration of blood supply to ischemic tissues may cause additional cell damage; i.e. reperfusion injury [46]. It should be noted that, during ischemia, redox centers and the pools of electron carriers, such as NAD(H), NADP(H), $FAD(H_2)$, ubiquinone (Q/QH_2) and glutathione (GSSG/GSH), become highly reduced. When O_2 is again available, the electron transport chain flow is restored; however, excessive reduction promotes the release of free radicals, which results in overproduction of reactive oxygen species (ROS) [47]. On the other hand, during reperfusion, the Ca^{2+} is accumulated within the injured tissue triggering additional overload, thus causing fatal cardiac arrhythmias and cell death [48]. The restored blood supply can also exacerbate the inflammation response, promoting white blood cells infiltration leading to necrosis and tissue destruction [49, 50].

## Lack of Estrogens Results in Cardiac Mitochondrial Dysfunction, Particularly After I/R

In an interesting work performed in 2000 [51], Zhai and co-workers reported morphological alterations in mitochondria from hearts subjected to ischemia/reperfusion, that were obtained from ovariectomized (OVX) female rats, fed with a diet without phytoestrogens and another group supplemented with 17β-estradiol. Such alterations included abnormal cristae and matrix cleared out (simulating vacuoles), in clear contraposition with the normal matrix and slight changes in cristae and shape observed in the OVX rats group that received a diet supplemented with 17β-estradiol [51]. These results reinforced the idea that estrogens have an important role in preserving mitochondrial structure and consequently protecting the myocardium against I/R injury.

In this respect, our group has established a clear association between the heart electrical activity, inflammation processes and mitochondrial function with the levels of sexual hormones in both female and male rats. We have observed that in male rats, castration does not affect the heart function, showing better electrocardiogram records; whereas in female rats the opposite was observed; i.e. removal of ovaries led to irreversible cardiac damage [52]. This sex-related difference was more evident when cardiac mitochondrial function was explored. In female rats, oophorectomy resulted in ~50% loss of mitochondrial OXPHOS coupling, characterized by a low respiratory control (RC) ratio (Intact RC = 6 ± 0.5 vs OVX RC = 3 ± 0.3); whereas in castrated male, the RC did not change significantly (Intact RC = 5 ± 0.8 vs Cast RC = 4 ± 0.9). The RC value not only reflects the intactness of the mitochondrial inner membrane after isolation but also the coupling between ATP synthesis and the respiratory chain activity [53]. It is calculated as the ratio between oxygen consumption rates

(OCR) in phosphorylating state (plus adenosine diphosphate (ADP) and phosphate; state 3) and in resting state (state 4); i.e. $RC = OCR_{state3}/OCR_{state4}$. Since the RC is a substrate-, tissue- and organism-dependent ratio, there is no absolute diagnostic value for mitochondrial dysfunction [54]. Nevertheless, it is widely accepted that higher RC values are representative of a more intact and "coupled"/functional preparation of isolated mitochondria. These results suggested that the OXPHOS pathway was altered in heart mitochondria from OVX female rats, but it is mandatory to explore these functional abnormalities further.

We have also found that mitochondria from OVX female rats were not capable to accumulate Ca^{2+}, in contrast with mitochondria from castrated males (Fig. 14.1) and that this effect was indeed time-dependent (Fig. 14.2) [55]. The mitochondrial Ca^{2+}-handling capacity was gradually lost after the second month post-oophorectomy (Fig. 14.2B). In general, mitochondrial Ca^{2+} accumulation is an important process regulating a variety of metabolic and pathological processes within cells [56].

In mammalian cardiac and skeletal muscle tissues, mitochondria occur as individual organelles, situated either in clusters beneath the sarcolemma (subsarcolemmal mitochondria, SSM) or in longitudinal rows within the contractile apparatus (interfibrillar mitochondria, IFM); thus occupying the entire space between Z-lines with usually one mitochondrion per sarcomere [57]. Overall, perinuclear mitochondria are smaller than interfibrillar and have a more rounded shape [58]. IFM are mainly situated in close proximity to the sarcoplasmic reticulum, which
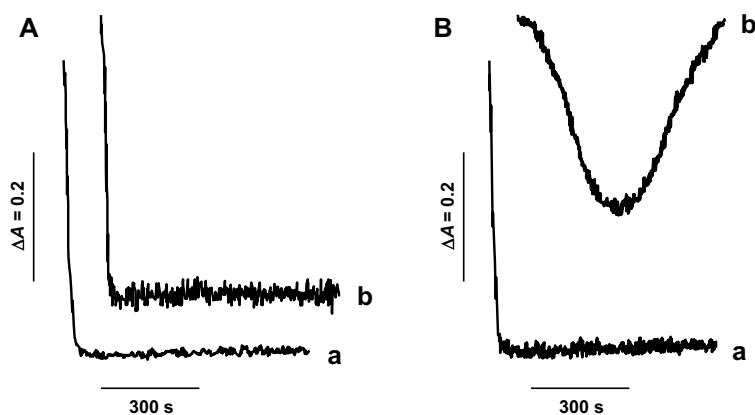
Fig. 14.1 Effect of castration/oophorectomy on Ca^{2+} uptake by heart mitochondria isolated from male and female rats. Mitochondrial Ca^{2+} uptake was monitored spectrophotometrically at 675–685 nm using the indicator Arsenazo III at 25 °C. To start the assay, mitochondrial protein (2 mg) was added into 3 mL of a medium containing 125 mM KCl, 10 mM succinate, 10 mM HEPES, 3 mM phosphate, 100 μM ADP, 5 μg rotenone, 50 μM Arsenazo III and 100 μM $CaCl_2$. (**A**); trace *a* shows Ca^{2+} handling in mitochondria from intact male rats; trace *b*, shows behavior in mitochondria from castrated male rats. (**B**); trace *a*, shows Ca^{2+} handling by intact female heart mitochondria; trace *b* shows failure in Ca^{2+} retention capacity in mitochondria isolated from OVX female rats. Representative traces from 10 independent experiments
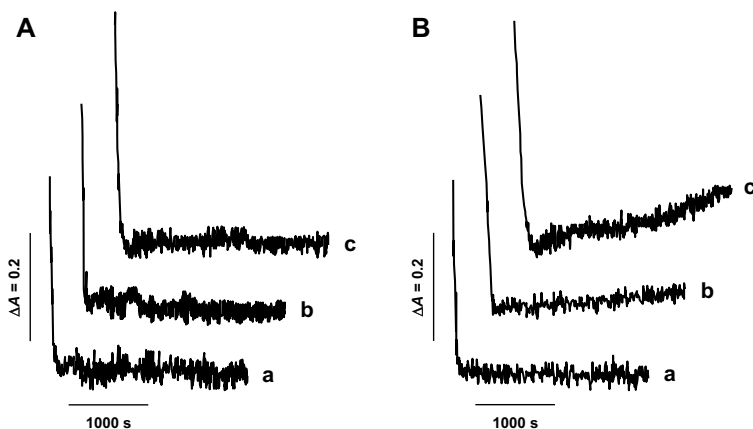
Fig. 14.2 Effect of oophorectomy on Ca^{2+} retention capacity by heart mitochondria isolated from female rats at different times post-surgery. Reaction mixture and assay conditions as described in Fig. 14.1. (**A**); traces *a, b, c* show Ca^{2+} uptake in mitochondria isolated from control female rats after 1, 2 and 3 months of sham surgery, respectively. (**B**); traces *a, b, c* show Ca^{2+} uptake in mitochondria isolated from OVX rats after 1, 2 and 3 months of ovaries removal, respectively. Note the clear impairment in Ca^{2+} retention that takes place at the third month post-oophorectomy (trace c). Representative traces from 10 independent experiments

stands between the IFM and T-tubule. This arrangement creates microdomains of high Ca^{2+} release; a.k.a. Ca^{2+} hotspots [59]. In other words, the correct function and location of mitochondria are critical for accurate Ca^{2+} cycling. The Ca^{2+} uptake in mitochondria is driven by the $\Delta\Psi_m$; thereby, both Ca^{2+} handling and OXPHOS processes should be tightly interconnected [60, 61]. In a related study, Ribeiro Junior and co-workers reported that the IFM and SSM exhibited a different susceptibility to estrogen deprivation [62]. These authors have also shown a lower ADP/O ratio in both subpopulations and a poor Ca^{2+} retention capacity in IFM from OVX rats. In addition, their studies showed that estrogen replacement restored the majority of the heart mitochondrial alterations induced by the ovariectomy.

The restoring effect of estrogens has been also demonstrated in human cell lines. After growing these cells in the presence of 17β-estradiol during 4–6 days, an increase in mitochondrial biogenesis and higher oxygen consumption were observed. In fact, a higher expression of different OXPHOS-related proteins occurred in 17β-estradiol-treated cells [63]. Similarly, our group observed a partial recuperation of the Ca^{2+} retention in isolated heart mitochondria from OVX female rats after being incubated with 17β-estradiol [52]. This effect was achieved by incubating the isolated organelles for ~2–3 h in the presence of the hormone (Fig. 14.3, trace c). Our results hence illustrate the direct effect estrogens have on mitochondrial function; however, further studies are essential in order to explain this phenomenon at the molecular level.

We have also shown that estrogens deprivation resulted in a progressive decrease in the expression and function of several aerobic metabolism-related proteins (Fig. 14.4) [55]. This situation was not dependent on testosterone. Protein content and enzyme

Fig. 14.3 Effect of 17β-estradiol on Ca^{2+} uptake by heart mitochondria isolated from OVX rats. Reaction mixture and assay conditions as described in Fig. 14.1. Trace *a*; Ca^{2+} retention by heart mitochondria isolated from female rats; trace *b* shows failure in Ca^{2+} retention capacity in mitochondria isolated from OVX female rats; trace *c* shows restoration of Ca^{2+} uptake in mitochondria from OVX females rats after being incubated with 17β-estradiol (100–300 nM) for ~2–3 h before the assay
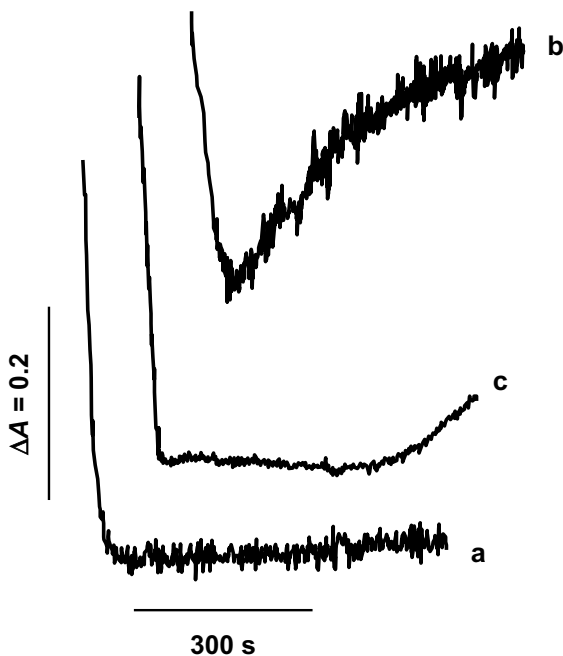
activities of respiratory complexes I (CI) and IV (CIV), pyruvate dehydrogenase (PDH) and 2-oxoglutarate dehydrogenase (OGDH) were clearly diminished in OVX female rats after the second month post-oophorectomy (Fig. 14.4). Although ~ 50% of CIV activity was lost (Fig. 14.4C), complex II (CII)-linked respiration and coupling did not change after estrogens deprivation. Conversely, both CI-linked respiration and coupling were severely affected. When electrons enter the respiratory chain through either CI or CII, the major flux control relies on complexes I/III or III/IV, respectively [64]. The stoichiometry for the respiratory complexes I:II:III:IV in heart mitochondria has been reported as 1:1.5:3:6–7 [65]; therefore, a partial decrease in CII and CIV contents would not be expected to modify the oxygen consumption activity as a CI deficiency would. It has also been described that the expression of genes encoding CI subunits ND1, NDUFS7 and NDUFS8 might be regulated by estrogens [66]. The latter may explain the decrease in cardiac CI content and activity in the OVX rats. Unexpectedly, expression of subunit ND1 of complex I did not change after oophorectomy (Fig. 14.4D). Subunit ND1 is a mtDNA-encoded protein, whereas subunits NDUFS7 and NDUFS8 are codified by nuclear genes. In this regard, the expression of nuclear-encoded subunits seemed to be more susceptible to estrogenic regulation since SDHC, COX4, glutaminase and PDH-E1α were markedly downregulated after oophorectomy. Moreover, it has been described that the regulation of glutaminase expression involves the estrogen-related receptor alpha (ERRα) during cell differentiation [67].
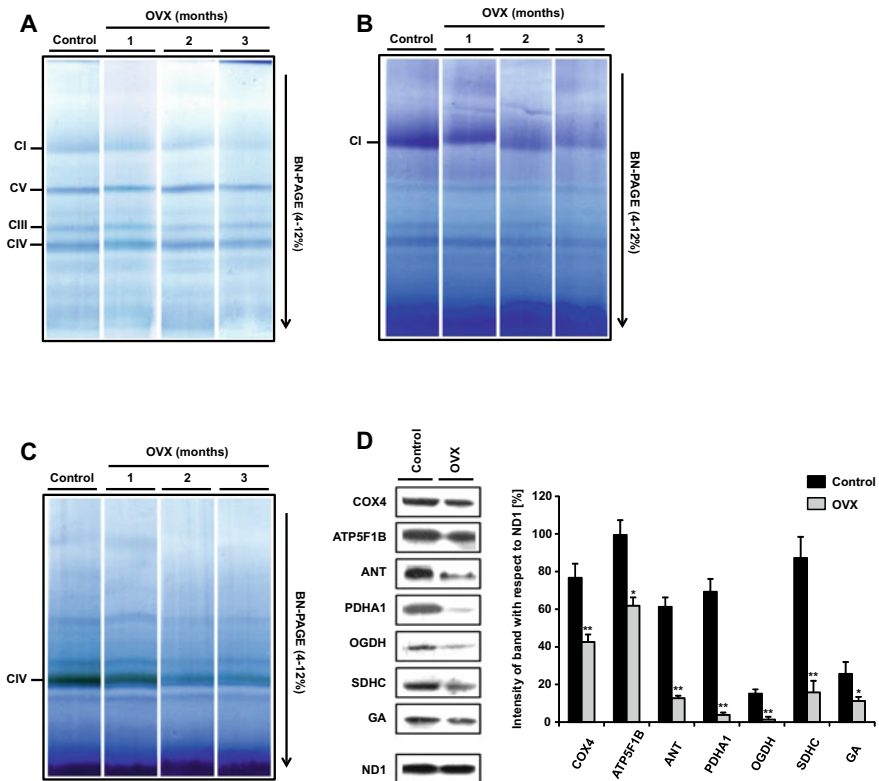
Fig. 14.4 Oophorectomy results in progressive decrease of several cardiac mitochondrial proteins and OXPHOS complexes. Isolated heart mitochondria from control and OVX (1-, 2- and 3-months post-surgery) rats were solubilized with dodecyl-maltoside (2 g/g protein) and separated by Blue Native-PAGE in 4–12% polyacrylamide gradient gels. (**A**); after electrophoretic separation, the proteins were stained with Coomassie blue dye. (**B**); In-gel NADH dehydrogenase activity staining for complex I was performed by incubating the gel in 10 mM Tris/HCl pH 7.0 supplemented with 1 mM NADH and 0.5 mg/ml Nitrotetrazolium blue chloride. (**C**); In-gel cytochrome *c* oxidase activity staining for complex IV was carried out by incubating the gel in sodium phosphate buffer pH 7.4 supplemented with 0.04% diaminobenzidine and 0.02% horse heart cytochrome *c*. OXPHOS complexes are marked as CI (complex I), CIII (complex III), CIV (complex IV) and CV (ATP synthase). (**D**); *Left panel*, western blot analysis of different mitochondrial proteins from control and OVX (3 months post-oophorectomy) female rat mitochondria. Analyzed proteins: ND1, subunit ND1 from complex I; COX4, cytochrome *c* oxidase subunit 4; ATP5F1B, ATP synthase subunit 5B (beta); ANT, adenine nucleotide translocase; PDHA1, pyruvate dehydrogenase subunit E1; OGDH, 2-oxoglutarate dehydrogenase; SDHC, succinate dehydrogenase subunit B; GA, glutaminase. *Right panel*, variations in relative content of analyzed proteins compared to the loading control (ND1). Representative blots and data from three independent experiments; *$p < 0.05$ and **$p < 0.01$

Estrogen Effects on Mitochondrial Permeability Transition and ROS Production After I/R

Mitochondrial impairments occurring in cardiovascular affections, such as myocardial hypertrophy, heart failure and ischemia/reperfusion could also trigger the opening of the so-called mitochondrial permeability transition pore (MPTP). As a result, molecules with masses up to 1.5 kDa can diffuse in a non-selective fashion across the mitochondrial membranes causing a high level of damage including large amplitude swelling, $\Delta\Psi_m$ dissipation, OXPHOS uncoupling, ATP depletion, exhaustion of glycolytic substrates, acidosis and cell death [68]. The mitochondrial permeability transition (MPT) has also been recognized as a key mechanism underlying both necrotic and apoptotic cell death [69]. However, the structure of MPTP remains controversial. On the one hand, it has been described that it is composed by the voltage-dependent anion channel (VDAC), phosphate carrier (PiC), adenine nucleotide translocase (ANT) and cyclophilin D (CypD) [70]. On the other hand, it has been recently proposed that the MPTP forms at the interface between complex V dimers [71].

Sex-related differences in mitochondrial permeability transition have been described in heart. In female rats, the Ca^{2+} retaining capacity of the mitochondrial matrix is higher than in male rats [72]. In addition, recovery of $\Delta\Psi_m$ after depolarization occurs faster, whereas the mitochondrial swelling after Ca^{2+} uptake is lower; hence, decreasing the possibility to trigger the MPTP opening [73, 74]. Remarkably, in hearts of male mice that were subjected to I/R, a brief exposure to 17β-estradiol favored the Ca^{2+} retention capacity by inhibiting the MPTP, which diminished the infarct size [75]. This cardio-protective effect might result from the interactions between CypD and the MPTP modulated by 17β-estradiol and ERβ [76]. Although the molecular mechanism is unclear, the combined effect of estrogens on the Ca^{2+} homeostasis and OXPHOS seems to prevent the MPTP opening after I/R. Since complex V and CypD levels are similar in both male and female hearts, it has been recently proposed that the lower sensitivity of the MPTP to the Ca^{2+}-induced swelling is also related to a higher ischemic tolerance of the female myocardium [77].

Besides, mitochondria are the major cellular source of ROS, i.e. H_2O_2, superoxide anion ($O_2^{\bullet-}$) and hydroxyl free radical ($^{\bullet}OH$). It has been estimated that 2% of the respiratory chain flux ends in formation of H_2O_2 [78]. Within mitochondria, the manganese-dependent superoxide dismutase (MnSOD or SOD2) converts $O_2^{\bullet-}$ to H_2O_2. Mitochondrial ROS may diffuse to the cytosol, where they participate in redox signaling pathways and other cell processes. However, ROS overproduction and failure of the antioxidant systems lead to oxidative stress and cell death. It is important to mention that estrogens control the expression of several antioxidant enzymes too, among them MnSOD, which has a higher expression in females [79]. Estrogens might upregulate ROS handling systems, thus protecting the female heart against oxidative stress. Until now two major ROS-forming sites at the OXPHOS system have been recognized: (*a*) the flavin mononucleotide (FMN) group of CI and

(*b*) the Q_O site in CIII [80]. Likewise, FAD-containing proteins, such as CII, PDH and OGDH, have been potentially suggested as ROS sources [81]. It has also been described that 17β-estradiol is capable to inhibit complex I at the FMN site [82]. Hence, it is proposed that this hormone may block the superoxide generation by CI, preferentially in females.

Other Cardiac Impairments Related to Estrogens Deprivation

The estrogen treatment has been shown to ameliorate and even prevent different levels of cardiac damage in several models of infarct, especially in vivo. Treating adult OVX mice subjected to coronary artery ligation with 17β-estradiol reduced the cardiomyocytes apoptosis, which is otherwise observed in untreated animals [83]. Similarly, OVX rabbits treated with estrogens prior to the induction of myocardial infarction, exhibited smaller heart stroke dimensions [84]. The estrogens effects observed in vitro are also remarkable; for instance, the presence of estrogens in cultures of adult rat myocytes obtained from infarcted hearts led to a lower degree of apoptosis and higher viability in comparison with the untreated controls [85]. Therefore, these observations deserve special attention since estrogens are able to activate different molecules involved in survival signaling pathways; e.g. Akt.

Akt (protein kinase B, PKB) is an important kinase which regulates not only apoptosis and cell proliferation but also a number of other cellular processes; such as, carbohydrates metabolism, motility and transcription. A higher activation of this kinase has been described in neonatal rat cardiomyocytes supplemented with 17β-estradiol [83]. The same effect has been also observed using an in vivo murine model; i.e. administration of 17β-estradiol before blocking the coronary artery promoted a high activation of Akt. In contrast, cardiac damage situations, such as pressure overload, result in a lower activation of Akt in OVX rats in comparison to the sham controls [86]. The latter reinforces the notion of a crucial connection among estrogens and Akt. In fact, it has been reported that cardiomyocytes from pre-menopausal women have a higher Akt activity than the one displayed by the same cells from men or post-menopausal women [87]. Certainly, all these findings strongly suggest that the cardioprotective effects of estrogens observed in the different cardiovascular disease models may also have a common molecular basis involving the regulation of the Akt activity/expression.

Moreover, estrogens and ERs have effects on the heart contractile function by controlling ion channels and indirectly the excitation–contraction (EC) coupling. The presence of 17β-estradiol in cultures of guinea-pig isolated ventricular myocytes reduces the peak inward Ca^{2+} current (I_{Ca}), which means that the hormone might have a Ca^{2+} channel inhibitory feature [88]. In another study using estrogen receptor-knockout (ERKO) mice [89], it has been observed an elongation in the action potential duration (APD), as well as a clear electrocardiographic difference; i.e., an increase in

the QT interval (~70%). In addition, the authors found an increased expression of the L-type Ca^{2+} channels resulting in a cardiac repolarization delay in ERKO mice [89]. When female rats were ovariectomized, it has been observed a higher expression of subunit Cav1.2 from L-type Ca^{2+} channels in ventricular myocytes [90].

Even so, estrogens not only affect calcium channels but also potassium (K^+) channels. It has been also noticed a downregulation of the subunits Kv1.5 and Kv4.3 in estrogen-treated OVX mice resulting in lower transient outward and delayed K^+ currents; i.e. APD is prolonged [91, 92]. These findings confirm again the existence of sex-related differences in the female heart and thus suggest that the signals triggered by estrogens are indeed important for Ca^{2+} and K^+ channels expression and optimal ion currents.

Perspectives for Elucidating the Molecular Basis of Sex Differences in Cardiovascular Disorders

Sex-related differences in cardiovascular diseases come about from a complex interaction of genetic, hormonal and environmental factors resulting in a profile of individual risk and phenotypic presentation of the disorder. Therefore, there is an enormous interest in elucidating all these interactions in order to optimize the patient treatments and outcomes. If estrogens were the only factors accounting for sex-related differences in the prevalence of ischemic heart disease, it might be expected an increase of this affection after the menopause and that estrogenic hormone replacement therapy would prevent it. The Women's Health Initiative study, however, found that the hormone replacement therapy did not reduce the risk of ischemic heart disease in postmenopausal women [93]. A recent large population-based prospective study found only a minor increase in the risk of ischemic heart disease after the menopause in 55–65 years old women [94]. These findings suggest that ovarian hormones are not the only molecular basis for the sex differences observed in heart; accordingly, we should continue looking for other factors in both males and females and their mechanistic roles in cardiac diseases.

The studies summarized on this chapter reveal pervasive sex-related differences in cardiovascular diseases (CVD) and document diverse mechanisms that contribute to their development. Since most of the studies have only focused on the role of gonadal hormones in cardiac disorders, the biological variable of sex has to be taken apart into its components to better understand the molecular mechanisms underlying those differences. As it has been reviewed within the previous specific sections, estrogens are the most frequently studied factors due to their cardioprotective effects. The shortage of studies about the roles of androgens in cardiac pathophysiology offers, however, an important opportunity for further investigations.

Interestingly, recent studies in gonadectomized mice with altered sex chromosomes have shown differences in I/R injured hearts related to the number and type of sex chromosomes [95, 96]. The cardiac infarct size after I/R in gonadectomized

adult mice with two X chromosomes (XX or XXY) is ~40–50%, larger than those containing only one X chromosome (XY or XO) [95]. In this case, the absence or presence of the Y chromosome had no effect; i.e. genes encoded in Y chromosome do not seem to contribute for sex-related differences in I/R injury. The presence of a second X chromosome was associated with adiposity which is a risk factor in the coronary heart disease [95]. Nevertheless, the effects on I/R injury were not related with this condition, since the damage occurred before the signs of adiposity become evident. In any case, it has been suggested that some putative genes of the second X chromosome encoding for proteins with metabolic function could be related to account for the sex differences in different metabolic phenotypes [97]. Therefore, this subset of genes escape inactivation since their expression is higher [98].

The effect of an additional X chromosome in female mice is paradoxical in I/R injury. In OVX female mice, it promotes higher damage instead of preventing it. This observation has a potential clinical significance in humans since female-specific factors may interact with one another resulting in a negative effect in women. In other words, the cardioprotective effect of 17β-estradiol could be mitigated by the presence of the second X chromosome. In women, the heart might be protected by the estrogens at early ages; but later, after the menopause, it would become more susceptible to I/R injury due to the deleterious effects of the second X chromosome [98].

The potential implication of sex chromosomes, especially X chromosome copy number, has only been reported in fundamental research studies. The particular genes involved, as well as their sites and mechanisms of action are still unidentified. In addition, both hormonal and sex chromosome functions clearly influence the same disease outcomes, but sometimes in opposite directions; again, the molecular basis for these interactions remains unclear. The specific sex-biasing factors in animal research have yet to be fully translated into the clinical field. However, the current evidence suggests that the effectiveness of specific drug therapies may depend on sex or the levels of sex-biasing factors, for instance estrogens [99].

Another research field that is emerging is related with the transsexual community, where men use estrogens by different ways of administration (transdermal, oral, injected, etc.) and in unknown doses; even up to 20 times the recommended doses for post-menopausal women. On the other hand, a similar situation happens in women who have chosen to change their gender and take supraphysiological doses of testosterone. It is urgent to perform more studies including these groups in order to prevent possible physiological alterations and damage. In this regard, a study from 2011 has found cardiovascular diseases, particularly ischemic heart disease, as one of the most frequent death causes in this community [100]. Accordingly, it is probable that we will observe more sex hormone-related effects in members of this group, which have not been described or even predicted from the laboratory research so far.

It is very interesting to notice that significant sex-related differences do exist not only in cardiovascular diseases but also in the basal heart function. For example, healthy women have higher ejection phase indices compared to healthy age-matched men. The effects of estrogens on mitochondrial and heart functions are unquestionably important and result from a combination of several genetic, metabolic and

signaling factors, which have a tremendous therapeutic potential in ameliorating the complications of cardiovascular diseases. Yet, administration of estrogens for this purpose is far from being safe and it must be still extensively tested in agreement with individual needs.

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