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REVIEW

Sex Differences in Cardiac Tolerance to Oxygen Deprivation – 40 Years of Cardiovascular Research

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Summary

Experimental and clinical studies have clearly demonstrated significant sex differences in myocardial structure and function, both under physiological and pathological conditions. The best example are significant sex differences in the cardiac tolerance to ischemia/reperfusion injury: pre-menopausal adult female hearts are more resistant as compared to the male myocardium. The importance of these findings is supported by the fact that the number of studies dealing with this issue increased significantly in recent years. Detailed molecular and cellular mechanisms responsible for sex differences are yet to be elucidated; however, it has been stressed that the differences cannot be explained only by the effect of estrogens. In recent years, a promising new hypothesis has been developed, suggesting that mitochondria may play a significant role in the sex differences in cardiac tolerance to oxygen deprivation. However, one is clear already today: sex differences are so important that they should be taken into consideration in the clinical practice for the selection of the optimal diagnostic and therapeutic strategy in the treatment of ischemic heart disease. The present review attempts to summarize the progress in cardiovascular research on sex-related differences in cardiac tolerance to oxygen deprivation during the last 40 years, i.e. from the first experimental observation. Particular attention was paid to the sex-related differences of the normal heart, sex-dependent tolerance to ischemia-reperfusion injury, the role of hormones and, finally, to the possible role of cardiac mitochondria in the mechanism of sex-dependent differences in cardiac tolerance to ischemia/reperfusion injury.

Key words

Female heart • Cardiac hypoxic tolerance • Ischemia-reperfusion injury • Sex differences

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Introduction

The most frequent (and hence the most widely studied) cardiovascular diseases of modern times undoubtedly include hypoxic states. They originate as a result of disproportion between the amount of oxygen supplied to the cardiac cell and the amount actually required by the cell. Degree of hypoxic injury depends not only on the intensity and duration of hypoxic stimuli but also on cardiac tolerance to oxygen deprivation. 40 years ago, in the study comparing cardiopulmonary responses of male and female rats to intermittent high-altitude hypoxia, we have observed significant sex differences in cardiac resistance to acute anoxia *in vitro* [1]. The myocardium of control adult female rats was significantly more resistant to oxygen deprivation as compared with males of the same age (Fig. 1). Adaptation to chronic hypoxia significantly increased resistance in both sexes, yet the sex difference was maintained. Unfortunately, our scientific interest was at that time concentrated on the protective mechanisms of cardiac adaptation to chronic hypoxia and the possible sex-dependent differences remained out of our research program. To our surprise, starting ten years later we have seen repeatedly information published in the high-quality journals that our paper from 1984 first described sex differences of myocardial resistance in female and male rats exposed to acute hypoxia [2-4]. Mistrust to this statement led us to the search for objective information:

and really, according to data from Web of Science, the number of studies investigating sex-related differences in the cardiovascular system was negligible still in 1989 [5] (Fig. 2). However, the number of clinical and experimental studies has grown exponentially over the past 30 years. This trend is obviously the result of several facts: the number of examples of different behaviour of the male and female heart under physiological and pathological conditions is steadily increasing and there are controversial reports on the beneficial and adverse effects of hormonal replacement therapy (HRT) in women during menopause. Moreover, the increasing interest undoubtedly reflects the importance of this topic and the urgent need to explain underlying mechanisms for better understanding sex determinants of outcomes and to minimize bias in the management and treatment of ischemic heart disease (IHD) in women.

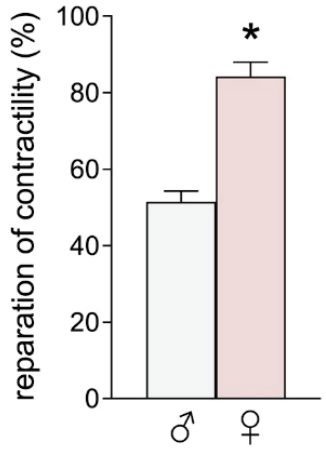

Fig. 1. Sex differences in the cardiac tolerance to acute oxygen deprivation in rats (expressed as % of the reparation of contractility of the isolated right ventricle after acute anoxia). *p<0.01; data from [1].

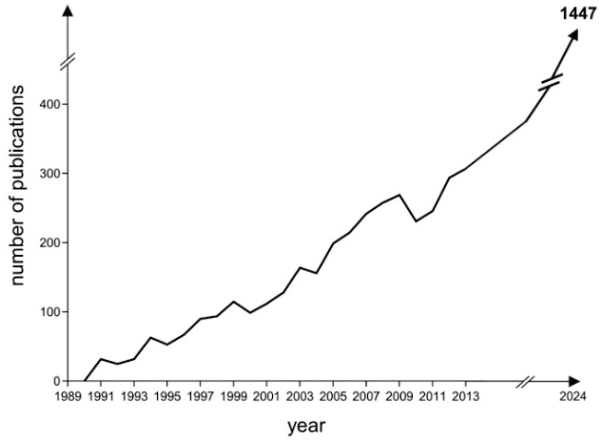

Fig. 2. Number of clinical and experimental papers dealing with „sex and heart AND female heart. From 1989 to 2024. Source: Web of Science.

The present review attempts to summarize the progress in cardiovascular research on sex-related differences in cardiac tolerance to oxygen deprivation during the last 40 years, i.e. from the first experimental observation. Particular attention was paid to the sex-related changes of the normal heart, sex-dependent tolerance to ischemia-reperfusion injury, role of hormones in sex-dependent variation in cardiac sensitivity to ischemia and, finally, to the possible role of cardiac mitochondria.

Sex differences of the normal heart

Sex-related cardiac differences are apparent even in healthy individuals (reviewed in [6]). Although there are no differences in the weight of the cardiac muscle during early phases of ontogenetic development, an increase in myocardial weight occurs in males at puberty; this change makes the male heart 15-20 % heavier than female heart [7]. The initial number of cardiomyocytes is comparable in both sexes; however, during ontogenetic development the number of cardiomyocytes in female hearts remains stable, whereas the number of myocytes in the male hearts decreases significantly [8]. The loss of cells is accompanied in the male myocardium by an increase in their diameter (by 51 % in male monkeys compared to 8 % in females [9]). This hypertrophic growth response can compensate to some extent for the decrease in the number of cardiac cells, but as the cells enlarge, the distance between the capillaries also increases, creating a potential source of insufficient oxygen supply to the cells. Surprising is the finding that the incidence of programmed cell death - apoptosis - is three times higher in the heart and coronary arteries of healthy men than in women; age did not influence this difference [10,11]. The average heart rate for women is approximately 3-5 beats/min more than for the males [12,13]. Moreover, the female heart has longer action potential duration, longer QT interval and a shorter sinus node recovery time as compared to the male heart [14]. In men under the age of 60 years, the average systolic and diastolic pressure is higher by 6–7mm Hg and 3-5mm Hg respectively, as compared to age-matched women. In post-menopausal women, the systolic blood pressure increases, to the extent that the incidence of hypertension is more prevalent in women than in men [15, 16].

Data on the myocardial contractile performance are controversial and not concise. For example, Schwertz

et al. [17 - 18] and Machuki *et al.* [19] have observed that female cardiomyocytes have a larger contraction and greater Ca^{2+} transient amplitude as compared to male cardiomyocytes, whereas Farrel *et al.* [20] have failed to confirm these findings. These contrasting observations were suggested by Machuki *et al.* [19] to be, at least partly, due to the use of whole ventricular myocytes versus left ventricular apical cardiomyocytes, particularly since differences in apical versus basal Ca^{2+} current have been reported in rabbit heart [21]. An important element of cardiomyocyte contraction is the cAMP-PKA-L-type Ca^{2+} channel pathway. Machuki *et al.* [19] have reported that intracellular cAMP, Ca^{2+} channel density and Ca^{2+} transient were larger in female than in male cardiomyocytes. These authors have also suggested that estrogen can regulate the expression of genes for the cAMP–L-type calcium channel pathway and contribute to sex differences in cardiac contraction.

Over the last years, the number of studies describing myocardial sex differences at the molecular and cellular level has increased (for rev. see [22]); their enumeration exceeds the possibilities of this review. For the purpose of this chapter, we have briefly summarized sex differences in cardiac calcium metabolism. It may be noted that Ca^{2+} homeostasis is regulated as a function of the estrous cycle [23], and myofilament Ca^{2+} density is increased in hearts of ovariectomized female rats. Interestingly, Ca^{2+} homeostasis is also regulated by testosterone, which activates phospholipase C and subsequent production of inositol-3-phosphate, which in turn mediates the release of Ca^{2+} from the sarcoplasmic reticulum and increases intracellular Ca^{2+} [24]. Higher expression of sarcolemmal and mitochondrial ATPsensitive potassium (KATP) channels has been reported in the female myocardium; their inhibition during ischemia increases the extent of tissue injury [25]. Estrogen regulates also the expression of phospholamban and ryanodine receptors. In this regard, the higher levels of ryanodine receptors in female cardiomyocytes are linked to higher Ca^{2+} release from the sarcoplasmic reticulum [26]. Interestingly, no sex differences have been observed in SERCA (Ca^{2+}-pump ATPase) expression level [18]. Compared to myocytes, little is known about cellular sex differences in the non-myocytes of the heart [21]: while cardiac myocytes constitute 70 %, they constitute only about 30 % of the total cell number.

Sex differences, with respect to cardiac structure, function and cellular mechanisms during aging, have been summarized by Keller and Howlett [27] and Sapp and Howlett [28]. Dworatzek *et al.* [29] observed agedependent sex differences also in myocardial collagen composition: type I, III, and VI collagens were significantly lower in aged female hearts. Similarly, Arellano *et al.* [30] revealed a specific down-regulation of sirtuins (Sirt1 and Sirt3) in aged female human hearts, which was accompanied by a decline in the mitochondrial anti-oxidative defense system.

Sex differences in cardiac tolerance to ischemia/reperfusion injury

The mentioned sex differences, characteristic of the normal myocardium, create a logical presumption of a possible different response of the heart muscle to various pathogenic stimuli, including ischemia/ reperfusion (I/R) injury. Among cardiovascular diseases, ischemic heart disease (IHD) is the single most frequent cause of death among men and women and is responsible for significant number from all cardiovascular events [31]. Even though IHD is the major cause of mortality in both women and men, it has largely been considered as a “male disease” and, therefore, the majority of experimental and clinical studies have been conducted in men. The information that women are discriminated in diagnostics and treatment of cardiovascular diseases was actually first indicated in the late 1980 [32], noting that women with signs of coronary artery disease required less intensive treatment than men. Another communication from the same research team [33] pointed out the problems associated with the indication of women for coronary surgical intervention. In the same year, the first comprehensive book on IHD in women was published [34].

Epidemiological studies have unequivocally demonstrated that in women before menopause, IHD begins about 10 years later than in men, and the occurrence of myocardial infarction is delayed by even 20 years. However, after menopause, the incidence of this disease increases more than 10 times in women, while in men of the same age it is only 4.5 times [35,36]. The cause are apparently sex differences in the development of atherosclerotic changes during development, which were already pointed out by Fejfar [37]; it is approximately the aforementioned 10 years; this fact is also supported by lower LDL-cholesterol levels and higher HDL-cholesterol values in postmenopausal women [38].

The vast majority of experimental studies confirm clinical observations (for an overview see [39,44]). As it has been mentioned in the Introduction, we found already 40 years ago [1] that the isolated right ventricle of the female laboratory rat is significantly more resistant to acute oxygen deprivation than the right ventricle of males. However, intensive research on this question began many years later. Higher resistance of female myocardium to I/R injury has been demonstrated in various species of laboratory animals (e.g. [24,44-46]). Females were found to have better recovery of contractile function and a lower incidence of reperfusion arrhythmias [47- 49]; Przyklenk *et al.* [50], however, did not observe this difference. Better functional recovery in females was accompanied by a smaller extent of ischemic damage, a lower level of lactate dehydrogenase and a lower production of inflammatory cytokines [51]. Similarly, transgenic females with increased expression of Na/Ca exchanger and β-adrenergic receptors [52,53] had less I/R injury and increased contractility compared to transgenic males. We have observed that sex differences in I/R injury also exist in spontaneously hypertensive rats: postischemic reparation of contractility was significantly higher in hypertensive females, despite the fact that the blood pressure level was comparable in both sexes [54]. Sex differences also exist in obese animals: infarct size was significantly larger in males than in females [55]. Experimental and clinical studies describe significant sex differences in remodeling after myocardial infarction [56-58]: in males, healing was slower with more frequent cardiac ruptures, apparently caused by premature degradation of the extracellular matrix by activation of metalloproteinases [57].

The development of cardiac resistance to oxygen deprivation has a characteristic ontogenetic development: after birth, the resistance of the hearts of male and female laboratory rats does not differ. From the beginning of sexual maturity, the resistance of the male heart decreases, while it does not change in females; thus, significant sex difference arises in adulthood [59]. It is interesting that interventions induced during early stages of ontogenetic development can significantly affect the resistance of the adult myocardium to ischemia in sex-dependent manner. We have observed that perinatal hypoxia increases the resistance of adult female heart to ischemia; on the contrary, in adult males was I/R injury significantly more expressed than in males kept under normoxic conditions [5,43,59] (Fig. 3) These results support the hypothesis that perinatal hypoxia represents a primary programming stimulus for the heart that may lead to sex-dependent sensitivity of the adult heart to ischemia. This fact may be clinically important in patients who have undergone a prolonged hypoxic period in the early stages of development, e.g. in children with hypoxemic congenital heart disease.

In this context, the question arises whether the high resistance of the female heart to hypoxia can be further increased by some of the known cardioprotective phenomena. However, the answer is not simple: the experimental work that dealt with this issue is rare and, moreover, not concise; we did not find clinical observations in the literature. We have observed that adaptation to chronic hypoxia increases cardiac resistance in both sexes; however, the sex difference observed in normoxic animals was preserved [1]. Data on the effect of ischemic preconditioning are contradictory: e.g. Humphreys *et al.* [61] observed the same degree of protection in male and female rats, whereas Wang *et al.* [62] failed to increase the resistance of female rabbit myocardium. Song *et al.* [63] found that the protective effect of preconditioning was lower in females than in males; similar conclusions were reached by Crisostomo *et al.* [64] in the case of ischemic postconditioning. Moreover, Lieder *et al.* [65] observed that sex is not decisive for the cardioprotective effect of pre- and postconditioning. The most plausible explanation seems to be the observation of Turcato *et al.* [66]: they did not find a protective effect of ischemic preconditioning in young females, whose resistance was primarily relatively high; with a decrease in tolerance to ischemia in older individuals, the effect of ischemic preconditioning appeared.

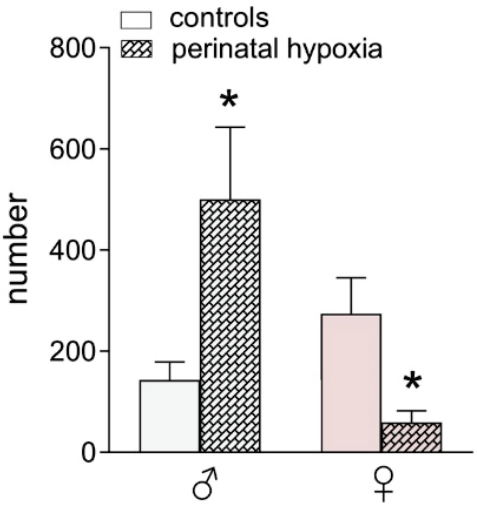

Fig. 3. Effect of perinatal hypoxia on the number of ischemic arrhythmias in adult males and females. * p<0.01; data from [60].

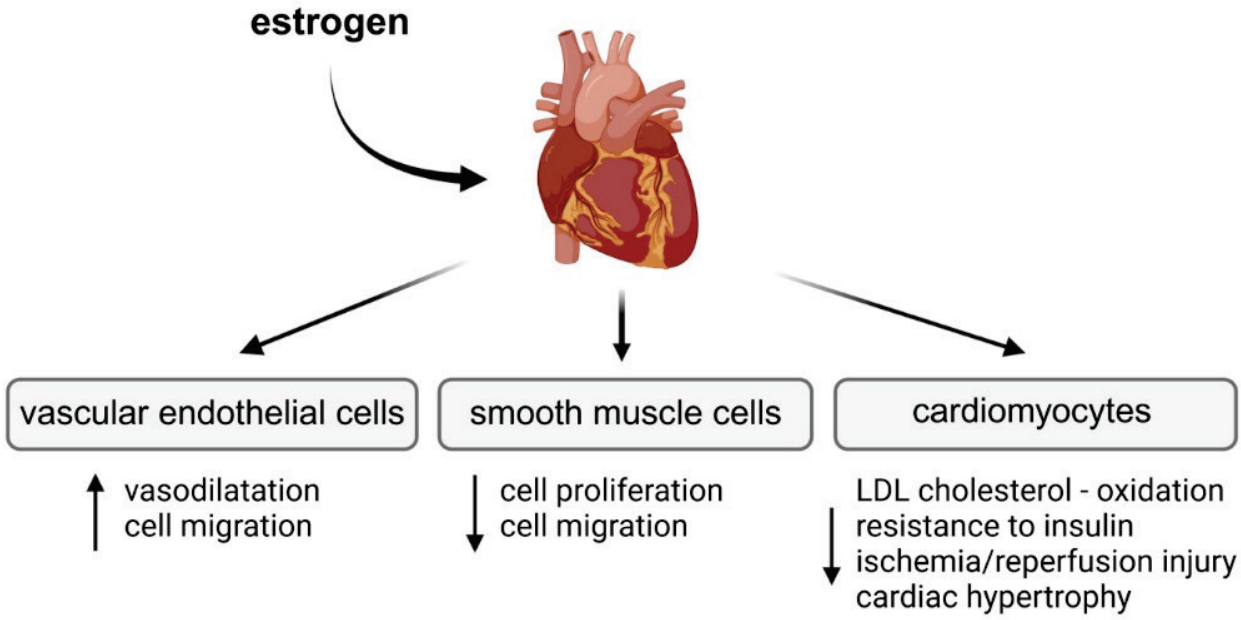

Fig. 4. Effect of estrogen on the heart; according to [68].

It seems to us that this observation belongs to the general biological phenomenon; the degree of cardiac resistance apparently has its threshold. Indeed, we observed a similar effect in the hearts of newborn rats; their high resistance could not be further increased either by adaptation to chronic hypoxia or by ischemic preconditioning; the protective phenomenon appeared only with a decrease in natural resistance during further stages of ontogenetic development [67]. In this context, it is necessary to recall the results of the CONDI-2/ERIC PPCI clinical study, which did not demonstrate a cardio-protective effect of remote ischemic preconditioning in patients with acute myocardial infarction, regardless of sex [68].

It follows that sex significantly affects cardiac resistance to I/R injury. However, we are still waiting for explanation of the pathogenetic mechanisms involved in this process. Let's try to briefly summarize the existing hypotheses.

Role of hormones in sex-dependent differences in cardiac sensitivity to ischemia-reperfusion injury

The most frequently mentioned cause of differences are sex hormones, especially estrogen. Its level changes during the ovarian cycle, during pregnancy, during hormonal contraception; it affects, among others, the function of blood vessels, the inflammatory response, the sensitivity of myocytes to insulin or the degree of development of cardiac muscle hypertrophy [69]. It is, therefore, understandable that experimental studies have focused on elucidating the role of estrogen in the cardiac tolerance to oxygen deprivation (Fig. 4).

There is clear evidence that ovariectomy in female rats increases the infarct size; on the contrary, the administration of estrogens has a protective effect on the male cardiac muscle [51]. Most of the protective effects of estrogens are attributed to their binding to estrogen receptors α and β, which have been demonstrated in female and male heart cells, fibroblasts and vascular smooth muscle [69,70], but are also found in cell membranes and mitochondria [71]. Their affinity for binding to 17βestradiol is the same in both sexes. Experimental studies show that these receptors play an important role in protection against I/R damage [72]. Unfortunately, there is still no consensus on which of the two receptors is responsible for the higher resistance of the female heart. However, there is a third membrane estrogen receptor, identified as G-protein-coupled estrogen receptor (GPER) [73]; it was found to inhibit the opening of the mitochondrial permeability transition pore (PTP) localized on the inner mitochondrial membrane [74]; the latter is involved in the development of ischemic damage (see later).

The binding of estrogens to receptors induces gene expression of a number of functional and structural proteins (the so-called "genomic effect"). In addition to the genomic effects, there are also the so-called "nongenomic" effects of estrogen; they occur rapidly and

independently of protein synthesis [75]. One of the many factors that can influence the response of the female myocardium is nitric oxide; its concentration is higher in female than in male myocardium. Blockade of NO synthase (eNOS) with L-NAME abolishes sex differences in susceptibility to I/R injury. It should be mentioned that a higher concentration of eNOS is also associated with Snitrosylation of L-type calcium channels, which significantly reduces I/R injury in females by decreasing the calcium overload of the cell [76]. In addition, estrogen activates phosphatidylinositol 3-kinase (PI3K) activity, which is considered to play a role in cardioprotection in females [77]. Taken together, it can be suggested that the protective effect of estrogen could be attributed to changes in the expression of specific proteins or altered post translational protein modifications. However, these are apparently not the only mechanisms involved in the cardiac ischemic protection in females. It seems that also e.g. sarcolemmal and mitochondrial KATP channels [78], higher activity of serine/threonine protein kinase (Akt), protein kinase Cε (PKCε) levels [79] or inhibition of proinflammatory tumor necrosis factor α (TNF α) in ischemic myocardium [80,81] may play a significant role. In all these considerations we must take into account the possible role of significant sex differences in cellular calcium metabolism, as discussed above.

The vast majority of experimental laboratories have chosen only one of the sex hormones - estrogen. It is clear that the cardiovascular system is influenced by at least one other powerful player, androgen. Both estrogenic and androgenic hormones are present in both sexes, although in different concentrations and ratios. Testosterone activates androgen receptors, which are expressed in myocytes; it increases the level of homocysteine and endothelin-1 and, by stimulating thyroxine hydrolase, increases the synthesis of catecholamines. Opinions on the effect of testosterone on cardiovascular function vary, both adverse and beneficial effects of testosterone on the heart have been reported [82,83]. It has been found that testosterone can increase the susceptibility to IHD in men [84], higher doses of androgenic steroids increased the development of atheroma [85]. However, there is no experimental evidence that physiological concentrations of testosterone induce myocardial ischemic damage. In contrast, other clinical work shows that testosterone can have a positive effect on the heart muscle [84]. This effect is apparently caused indirectly by conversion to dihydrotestosterone or 17β-estradiol. It was found, for example, that administration of testosterone to ovariectomized females reduced the extent of ischemic damage [86]. Furthermore, Ghimire *et al.* [87] showed that low doses of testosterone have a protective effect against I/R injury in older mice. Cavasin *et al.* [88] demonstrated in a mouse model of myocardial infarction that whereas estrogens prevent maladaptive chronic remodeling and further deterioration of cardiac performance, testosterone adversely affects myocardial healing (as indicated by a higher rate of cardiac rupture), and thus contributed to cardiac dysfunction as well as to adverse cardiac remodeling. On the other hand, Tsang *et al.* [89] observed that testosterone conferred cardioprotection by up regulating the cardiac α1adrenoceptor; this beneficial effect was abolished or attenuated by blockade of androgen receptors. These conflicting results obviously need further experimental analyses under precisely defined and thus comparable conditions: experimental model, form of steroid hormone, dosage, timing and evaluation. It is, however, necessary to stress, that precise understanding is complicated also by the fact that steroid hormone receptors do not act alone but interact with a broad spectrum of co-regulatory proteins to alter transcription [90].

Possible role of mitochondria

The number of different hypotheses trying to explain the causes of sex differences in the cardiac resistance to oxygen deficiency is increasing. In recent years a new promising opinion has appeared, suggesting that mitochondria, organelles responsible for oxygen handling, may be significantly involved in this effect [91-93]. Mitochondrial sexual dimorphism has been described in a number of organs such as liver, heart, brain and adipose tissue.

Cardiomyocytes from female rats exhibit lower mitochondrial content, but are more efficient and more differentiated than male mitochondria [94]. Moreover, they generate less reactive oxygen species (ROS) than male ones and have higher capacity of antioxidant defence [51]. At baseline, no difference in oxygen consumption rate and cardiolipin content is observed between mitochondria from male and female rats [95]. Subsarcolemmal and intermyofibrillar isolated mitochondria from female hearts have the same respiration rates as the male ones except for glutamate-malatestimulated respiration which is lower in females,

while the ADP/O ratio is higher [96]. 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 [92]. Recently, Cao *et al.* [97] have observed that cardiac mitochondrial DNA levels and function tend to be reduced in females as compared to males; on the other hand, the expression of genes, encoding mitochondrial proteins, are higher in males than females.

Murphy and Steenbergen [45] suggested that mitochondria are major targets of cardioprotective signalling. Lagranha *et al.* [51] have observed that cardioprotection in females was associated with altered mitochondrial proteins. They found that mitochondria isolated from females exhibited a number of post-translational modifications in mitochondrial enzymes involved in regulating the generation of ROS and oxidative metabolism. Therefore, females exhibit reduced ROS generation and oxidative metabolism. Morkuniene *et al.* [98] and Pavón *et al.* [99] described the relevance of estrogens in maintaining proper mitochondrial function in response to the instability of mitochondrial membrane potential and PTP opening after I/R. They observed that the opening of this pore can be blocked by physiological concentrations of estrogens, similar to blockade with the classic inhibitor cyclosporine.

Significant sex differences were also found in the mitochondrial uptake of Ca^{2+}: mitochondria from female hearts have lower Ca^{2+} uptake rates and improved recovery of mitochondrial membrane potential from Ca^{2+} - induced depolarization [100]. They cope more successfully with external calcium load by decreasing the rate of calcium influx by the calcium uniporter (MCU). The interaction between MCU and calcium uptake regulatory proteins M1CU1, M1CU2, MCUR1, SLC25A23, and EMRE may be here of crucial importance [101]. In addition, Chweih *et al.* [102] have observed that the concentration threshold for net mitochondrial Ca^{2+} uptake was higher in the female heart than in male myocardium. All these findings suggest that female heart mitochondria are less prone to Ca^{2+} overload upon its effect [103-105].

It has been known for a long time that mitochondria become leaky, uncoupled, and massively swollen if they are exposed to high Ca^{2+} concentrations, especially in the presence of phosphate and when accompanied by oxidative stress. The collapse of mitochondrial membrane potential due to opening of permeability transition pore (PTP), localized on the inner mitochondrial membrane, has been implicated in the molecular mechanism of cardiac I/R injury [106,107]. PTP is closed during ischemia due to the low pH (<7.0), but it opens during the first minutes of reperfusion, together with normalization of pH, ROS accumulation, and rise in intracellular calcium. PTP opening accompanied by matrix swelling, leads finally to myocardial cell death [107]. Initial support for the role of PTP in I/R injury was provided by pharmacology: the blockade of PTP by cyclosporine A and sanglifehrin A in perfused heart was cardioprotective in most animal models of cardiac I/R injury [108]. Cyclosporine A was cardioprotective also in small groups of patients with myocardial infarction undergoing percutaneous coronary intervention [108]. However, a large multicenter clinical trial (CIRCUS) revealed no protective effect of cyclosporine A on clinical outcome in patients with myocardial infarction [110,111]. Several factors such as the severity of infarction, a quite narrow window of protection, route of application, and timing of administration as well as comorbidities may be responsible for the lack of cardioprotection in the CIRCUS trial. Nevertheless, these studies challenge the clinical use of cyclosporine A and the possible cyclophiline D (CypD) inhibitors for cardioprotection, and emphasize the importance of further studies to clarify whether CypD is a feasible target for inhibition that can protect the heart from I/R injury [112].

We have tested the hypothesis whether the role of mitochondrial PTP in the pathogenesis of I/R damage to the heart muscle is dependent on sex [42,93,113]. We found that cardiac mitochondria of females are significantly more resistant to swelling induced by higher calcium concentration, indicating their greater resistance to MPTP opening (Fig. 5). Since the opening of the pore is closely related to the development of I/R damage, the higher resistance of this structure to calcium is one possible explanation for the higher tolerance of the female heart. In this context, the question arises as to whether the protein composition of PTP is responsible for these sex differences. Our experiments showed that there is no sex difference in substrate oxidation or ATP formation, which indicates a comparable content of respiratory chain enzymes. This observation was confirmed by quantitative immunodetection: female and male mitochondria contain comparable amounts of ATP synthase (the protein complex responsible for mitochondrial PTP function), as well as the regulatory

protein cyclophilin D. Interestingly, we observed similar results in our previous study, comparing the role of the mitochondrial PTP in highly hypoxic resistant neonatal and adult hearts of laboratory rats [114]. Therefore, it seems that the protein composition of mitochondrial PTP is not responsible for sex differences in cardiac tolerance to oxygen deprivation, but rather reflects sex differences in the regulation of its function, probably together with regulation of CypD by posttranslational modifications [109]. Cyclophilin D thus remains an attractive target for both experimental and clinical studies looking for possible mitochondrial PTP blockers as a way to reduce myocardial I/R damage [115]. It may be, therefore, concluded that mitochondria are significantly involved in the mechanism of sex differences in cardiac tolerance to I/R injury.

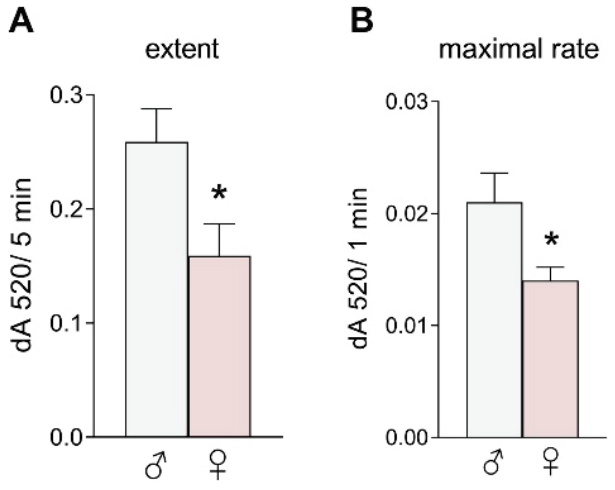

Fig. 5. Calcium induced swelling by rat heart mitochondria from male and female rats. (**A**) Extent of swelling was calculated from the swelling curves and expressed as the decrease of absorbance at 520 nm during 5 min after addition of 200 μM $CaCl_2$. (**B**) Maximum rate of swelling was calculated from curves obtained after derivatization of data of the extent of swelling. * $p<0.01$; data from [113].

Sex differences today

Unfortunately, despite a growing body of evidence, the distinct contribution of biological sex and the sociocultural dimension of gender to the manifestations and outcomes of IHD remain unknown. Moreover, the relative contribution of purely biological factors, such as genes and hormones, to cardiovascular phenotypes and outcomes is not yet fully understood [116]. In spite of the increasing awareness of sex differences in the management of patients with IHD in Europe, a recent study by Hellgren *et al.* [117] confirmed that women still receive guideline-recommended therapies less often than men. Sex-based disparities in outcomes and quality of care were summarized by Aggarwal *el al.* [118]. They include higher morbidity and mortality, delayed presentation, fewer revascularization, less cardiac rehabilitation and less intense pharmacotherapy in females. Recognition of sex differences in presentation, pathophysiology, treatment and outcomes accentuates the need for sex-specific research. Underrepresentation of females results in male outcomes being extrapolated to females, which does not consider sex and gender differences. In conclusion, although women develop IHD later in life than men, the underestimation of women-specific IHD pathophysiology, including biological and sociocultural components, the lack of early recognition and the lack of women-specific treatments increase the risk and mortality of IHD in women [40,116].

Similarly, sex differences are relatively understudied also in animal experiments; many studies fail to report the sex of the cells also in *in vitro* experiments. Moreover, most of experimental studies use exclusively males [40]. On the other hand, it is necessary to admit that experimental approach contributed significantly to our present knowledge on the mechanisms involved in the sex differences of the normal and ischemic heart. The observation that cells from males and females are inherently different is becoming increasingly clear – either due to acquired differences from hormones and other factors or due to intrinsic differences in genotype (XX or XY). In myocardial diseases, sex differences have been described at the tissue level [119]. However, in cells obtained from adults it is difficult to distinguish genetically determined sex differences that exist at birth from sex differences developed during the disease course and are the result of hormones or the environment.

A significant progress in our understanding of the development of sex differences brought the promising studies published by Shi *et al.* [120] and Deegan *et al.* [121], demonstrating that sex chromosome-specific differences in cardiomyocytes exist even before gonads are activated in the embryo. This finding confirm that cardiac sex-related disparities can occur at the early stages of heart development, before gonad formation, and are therefore independent of the influence of sex hormones or the environment. Moreover, identifying how hormones influence sex chromosome effects, whether

antagonistically or synergistically, will enhance our understanding how sex disparities are established. These studies support the view that purely biological mechanisms – genes and sex steroids - contribute to sexrelated differences in IHD and thereby emphasize the importance of sex-specific experimental research on human disease [116].

Conclusions

It follows from the data available that male and female cardiovascular system differ significantly in many characteristics under both physiological and pathological conditions. These differences should be considered by the selection of optimum diagnostic and therapeutic procedures in clinical practice. However, their detailed mechanisms are still poorly understood and the evidence available to date regarding sex-specific aspects of management and outcomes in cardiovascular diseases is still rather limited. Nevertheless, one is clear already today: sex differences in cardiac tolerance to ischemic injury are so important that they should be taken into consideration both in experimental and clinical cardiology.

Conflict of Interest

There is no conflict of interest.

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