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Female cardiovascular biology and resilience in the setting of physiological and pathological stress

Helen E. Collins

Center for Cardiometabolic Science, Christina Lee Brown Envirome Institute, Division of Environmental Medicine, Department of Medicine, Delia B. Baxter Research Building, University of Louisville, 580 S. Preston S, Louisville, KY 40202, USA

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ABSTRACT

For years, females were thought of as smaller men with complex hormonal cycles; as a result, females have been largely excluded from preclinical and clinical research. However, in the last ten years, with the increased focus on sex as a biological variable, it has become clear that this is not the case, and in fact, male and female cardiovascular biology and cardiac stress responses differ substantially. Premenopausal women are protected from cardiovascular diseases, such as myocardial infarction and resultant heart failure, having preserved cardiac function, reduced adverse remodeling, and increased survival. Many underlying biological processes that contribute to ventricular remodeling differ between the sexes, such as cellular metabolism; immune cell responses; cardiac fibrosis and extracellular matrix remodeling; cardiomyocyte dysfunction; and endothelial biology; however, it is unclear how these changes afford protection to the female heart. Although many of these changes are dependent on protection provided by female sex hormones, several of these changes occur independent of sex hormones, suggesting that the nature of these changes is more complex than initially thought. This may be why studies focused on the cardiovascular benefits of hormone replacement therapy in post-menopausal women have provided mixed results. Some of the complexity likely stems from the fact that the cellular composition of the heart is sexually dimorphic and that in the setting of MI, different subpopulations of these cell types are apparent. Despite the documented sex-differences in cardiovascular (patho)physiology, the underlying mechanisms that contribute are largely unknown due to inconsistent findings amongst investigators and, in some cases, lack of rigor in reporting and consideration of sex-dependent variables. Therefore, this review aims to describe current understanding of the sex-dependent differences in the myocardium in response to physiological and pathological stressors, with a focus on the sex-dependent differences that contribute to post-infarction remodeling and resultant functional decline.

1. Introduction

Cardiovascular diseases are the leading cause of death worldwide in both men and women [1]. Characteristically, premenopausal women are protected from adverse cardiovascular events, which is thought to be, in part, the result of the protection afforded by female sex hormones, such as estradiol (E2) [2]. It is also evolutionarily advantageous for females at childbearing age to be protected from cardiovascular stress so that they may go on to have children. However, with advancing age and the onset of menopause, females exhibit a heightened risk of developing cardiovascular disease compared with age-matched males, which is often not rescued by hormone replacement therapy, indicating contributions from other processes. Furthermore, in the clinic, several cardiovascular therapeutics have reduced efficacy and effectiveness in females due to prior clinical testing in males. Sex differences exist in the prevalence of several cardiovascular diseases, including myocardial infarction (MI), hypertension, hypertrophic cardiomyopathy (HCM), and dilated cardiomyopathy (DCM), with females having both a reduced disease and mortality risk compared with age-matched males. Several underlying processes contribute to sex differences in cardiac remodeling, such as changes in calcium signaling, electrophysiology, metabolism, inflammation, fibrosis, apoptosis, and sex hormones, as well as innate genetic differences caused by X and Y chromosomes. In addition, sex differences have been observed in terms of the cellular composition of the heart, and male and female cardiac cell types have been shown to have different genetic enrichment patterns and functions. Most of these sex-specific cardiovascular findings have only come to light in recent years with the implementation and additional consideration of “sex as a biological

E-mail address: Helen.collins@louisville.edu.

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variable" in preclinical and clinical studies. This is outlined in Fig. 1, where the number of publications describing sex differences in cardiovascular disease is plotted. Advancement was primarily hampered by the predominant focus of cardiovascular research on male subjects, often with female subjects excluded from preclinical and clinical studies due to the perceived complexities associated with hormonal cycling and the general cardioprotective nature of the female sex hormones in premenopausal women, resulting in reduced disease risk. As a result, there remains relatively little knowledge of the intrinsic biological and metabolic changes that occur in the female heart under physiological conditions and how the female heart responds to cardiac stressors. Therefore, we discuss the current understanding of sex-based differences in the heart with physiological and pathological stress, the latter focusing on post-myocardial infarction remodeling. We also discuss potential mechanisms for increased female cardiac resilience in this setting. Although sex-dependent differences are systemic in nature (see the following reviews and publications that provide an overview of the documented systemic differences observed in other organ systems, such as the liver [3–5], kidney [6,7], muscle [5,8,9], lung [10,11], and within the circulation [12,13], we focus this review exclusively on sex-dependent differences in the cardiovascular system.

2. Sex differences in normal cardiac physiology

For years, females were often thought of as smaller men; however, it has become clear that this is not the case. Several sex-dependent differences have been documented regarding basic cardiovascular structure and function. Men have larger hearts, with thicker ventricular walls and chamber dimensions than female counterparts [14], which contributes to increased stroke volume in males. Cardiac output is comparable between sexes due to increased heart rates in the female [15]. Although the difference in heart size may be expected given that males often have larger body weights compared with females, these differences occur irrespective of this because sex differences are still present in studies that have compensated for changes in lean body mass [16,17]. Female hearts also exhibit increased stiffness versus male hearts with exercise [18–20], which is further increased with aging and with cardiovascular disease (CVD) [18], and females tend to have increased ejection fraction in comparison to men [21]. A recent study also documented that in addition to increased blood volumes, females have increased myocardial perfusion compared with males [22]. Unlike cardiac mass, both body weight and sex hormones play a critical role in blood pressure differences between sexes, with males having higher systolic and diastolic blood pressures than females [17,23]. More recently, differences in X and Y chromosomes have been shown to contribute to changes in blood pressure because studies using the four core genotypes model (i.e., XY$^-$ and XX female and XY$^-$*Sry* and XX*Sry* male mice) revealed that Angiotensin II-mediated increases in blood pressure were greater in XX mice irrespective of sex [24,25]. Sex-dependent changes in basic cardiovascular physiology are shown in Fig. 2. These studies suggest that significant sex-dependent changes exist in normal cardiovascular structure and function, independent of cardiac stress, which could ultimately shape the responses of both sexes to stress.

2.1. Sex differences in the cellular composition of the heart and cardiac cell function

In recent years, with the increased technological advances with single-cell RNA-seq, it has become evident that the heart is not only composed of different cell types but that these cell types differ in number and function between the sexes. Analysis of cell populations in the human heart revealed that female hearts have relatively more cardiomyocytes and that male hearts had higher numbers of endothelial cells [26]. Mouse studies have shown similar findings regarding the numbers of both cardiomyocytes and endothelial cells in the hearts of male and female animals [27]. The murine heart also exhibits sex-dependent differences in mesenchymal-derived cells and immune cell populations [27]. Female hearts have increased numbers of fibroblasts, pericytes, and smooth muscle cells and reduced numbers of leukocytes versus age-matched male hearts [27]. These differences were shown to be dependent on both male and female sex hormones. However, even though cellular sex is now a recognized biological variable, this is often not reported in many preclinical studies, so the sexual origin of the cells used in many studies is often unclear. These studies indicate

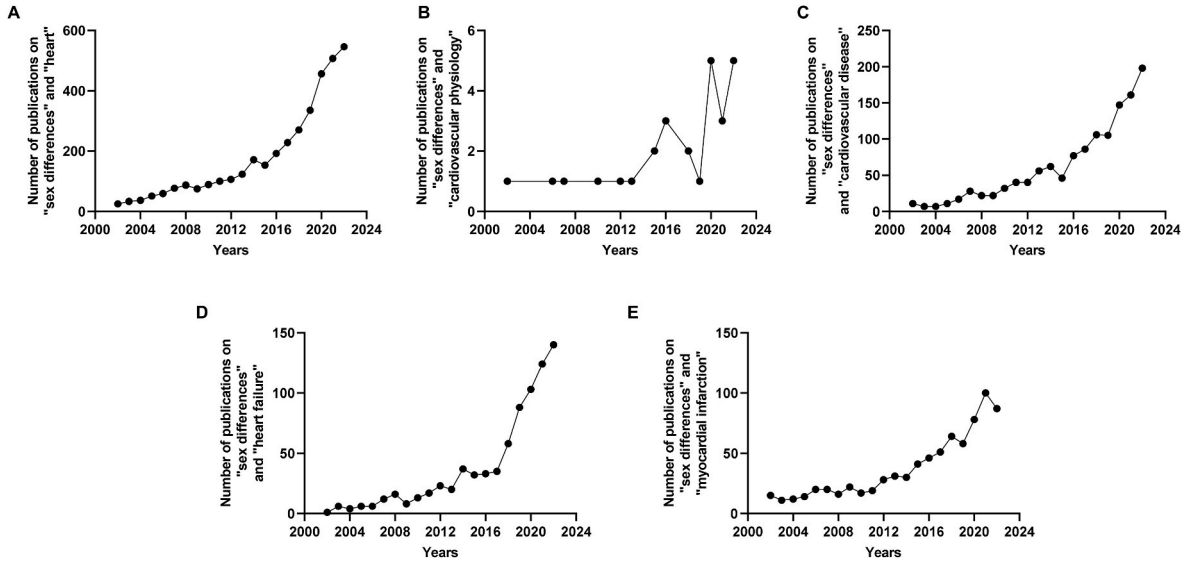

Fig. 1. Number of publications investigating sex differences in cardiovascular physiology and disease. (A) This line graph was composed using data obtained from PubMed analytics using the following key search words: "sex differences" and "heart" between 2002 and 2022. (B) This line graph was composed using data obtained from PubMed analytics using the following key search words: "sex differences" and "cardiovascular physiology" between 2002 and 2022. (C) This line graph was composed using data obtained from PubMed analytics using the following key search words: "sex differences" and "cardiovascular disease" between 2002 and 2022. (D) This line graph was composed using data obtained from PubMed analytics using the following key search words: "sex differences" and "heart failure" between 2002 and 2022. (E) This line graph was composed using data obtained from PubMed analytics using the following key search words: "sex differences" and "myocardial infarction" between 2002 and 2022. All searches were made in March 2023.

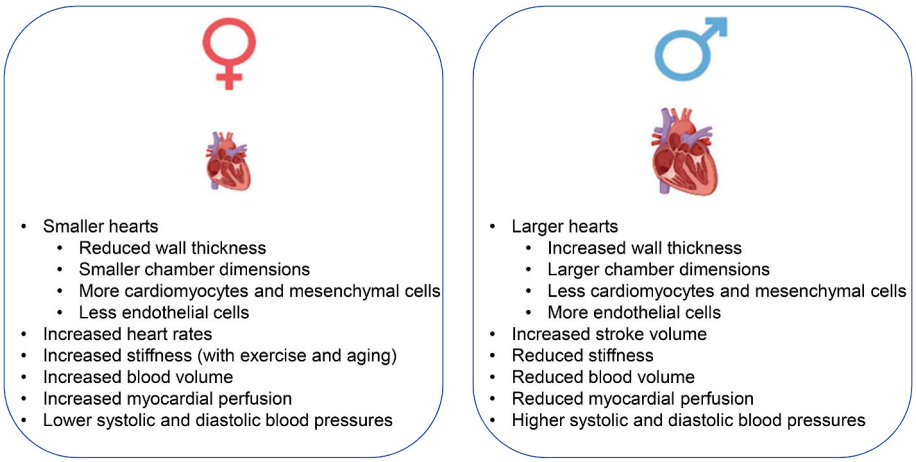

Fig. 2. Sex-dependent differences in basic cardiovascular physiology. This schematic shows the major differences in cardiac structure and function between sexes. Female hearts are often smaller than male hearts, with reduced chamber dimensions, and reduced stroke volumes. Female hearts also exhibit increased ejection fraction, increased blood volume and myocardial perfusion and reduced blood pressures when compared to men.

that there are significant sex-dependent differences in the cellular composition of the heart, which are likely to play a role in how male and female hearts respond to insult or injury. Nevertheless, few studies have examined how these innate differences influence remodeling of the heart, which is complicated further by the emergence of cellular subpopulations [26] in conditions such as MI [28]. The implications of these changes are discussed in subsequent sections in the contexts of post-infarction remodeling and physiological remodeling.

2.2. Sex differences in response to physiological stressors

There is an emerging body of evidence that suggests that there are sex-dependent differences in how the heart responds and adapts to physiological stressors, such as exercise. In response to acute moderate-to-high intensity treadmill exercise in male and female FVB/NJ mice, Fulghum et al. [29] showed increased amino acid metabolites (e.g., branched-chain amino acids, serine, alanine, tyrosine, and tryptophan) and 3-hydroxybutyrate in female hearts compared with male hearts; these changes were associated with higher basal sensitivity of female mitochondria to adenosine diphosphate. Interestingly, a larger number of significant sex-dependent changes were observed in the cardiac metabolome of sedentary mice, indicating that the cardiac metabolome is impacted by sex independent of physical activity. Notably, many of these metabolic changes in the female heart occurred independently of cardiac growth. Additional studies have shown that cardiac AMP-activated protein kinase activity is higher in male mice in comparison with female mice in response to exercise training [30], and changes in cardiac substrate utilization between sexes have been implicated in exercise-induced hypertrophy, with females having higher fatty acid oxidation than males, with a more significant increase in cardiac mass [31]. In addition, trained female hearts have increased Ca^{2+}/calmodulin-dependent protein kinase activity and sustained phosphorylation of glycogen synthase kinase 3β compared with trained male hearts, which was associated with greater exercise-induced hypertrophy [32]. These studies suggest that cardiac adaptations to exercise are sexually dimorphic due, at least in part, to differences in metabolic remodeling. However, additional studies are required to interrogate thoroughly metabolic flexibility and substrate utilization between male and female hearts. Regarding exercise, which impacts systemic physiology, changes in organ-organ communication and circulating metabolites could contribute significantly and should be examined in future studies.

Premenopausal women often undergo additional physiological cardiac stress in the form of pregnancy within their lifetime. Normal pregnancy is associated with the development of pregnancy-associated hypertrophy that arises to meet maternal and fetal circulatory demands, which resolves following birth [33–35]. This resolution occurs upon reversal of hemodynamic changes that occur during pregnancy and following lactation. Similar findings are recapitulated in rodent pregnancy studies [36–40]. Women who have had normal, uncomplicated pregnancies have greater cardiovascular protection later in life in response to stressors, such as MI; however, this is primarily the case for women who choose to breastfeed [41,42]. On the other hand, females with pregnancies complicated by cardiovascular diseases, such as pre-eclampsia and peripartum cardiomyopathy, have increased all-cause mortality and cardiovascular mortality rates later in life [43], suggesting that cardiovascular disease during pregnancy can have a long-term impact on cardiovascular health. Despite this, pregnancy is often not considered a cardiovascular risk factor in preclinical and clinical research. In fact, many preclinical studies that examine responses of the heart to pathological stress do not include animals that have previously been pregnant, often opting for virgin females, which is not representative of what happens in nature and may impact the translational nature of sex-dependent observations. In addition, few preclinical studies have examined the long-term benefit of lactation in the setting of cardiovascular diseases, such as MI. Overall, these studies suggest that not only do males and females have different cardiac responses to physiological stress but that it seems as though little has been determined in these situations regarding the potential underlying mechanisms.

3. Sex differences in cardiovascular diseases

There are documented sex differences in the prevalence, presentation, and outcomes of cardiovascular disease (CVD), highlighted in Fig. 3. Premenopausal females are protected from the development of many cardiovascular diseases. For example, compared with males, premenopausal females have a reduced risk of developing coronary heart disease [44], hypertension [24], MI [45], genetic cardiomyopathies (e.g., HCM and DCM) [46,47], heart failure with reduced ejection fraction (HFrEF) [48], and the long-term cardiovascular complications associated with COVID-19 [49]. Regarding the documented sex differences in the presentation of several CVD, females often exhibit different symptoms compared with males, such as those unrelated to chest pain, making prompt diagnosis and timely yet effective treatment problematic because women delay seeking treatment [50]. This likely contributes to increased mortality in females following acute MI [51]. In addition, some of the associated risk factors for developing CVD between the sexes

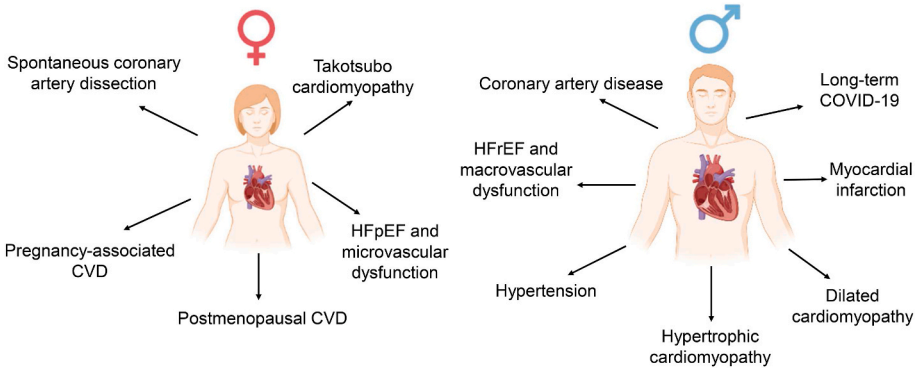

Fig. 3. Sex-dependent differences in cardiovascular diseases. This schematic highlights the predominant female and male cardiovascular diseases. Premenopausal women are less prone to the development of coronary artery disease, hypertension, myocardial infarction, and heart failure with reduced ejection fraction. However, women are more likely to develop diseases associated with emotional stress, changes in hormonal status, and as a consequence of female specific risk factors, such as prior CVD in pregnancy.

are also different, which likely contributes to inconsistencies in underlying symptoms and clinical presentation. For example, females often have unique risk factors such as pregnancy-associated cardiovascular complications (e.g., hypertensive disorders of pregnancy), oral contraceptive use, menopause, and hormone replacement therapy, which increase the risk of MI with age. With the onset of menopause, females begin to catch up with males, having comparable and in some cases increased CVD risk. In fact, many instances in which females are more susceptible to CVD occur upon the decline in E2 associated with the onset of menopause or conditions of emotional stress. For example, postmenopausal females are more likely to develop heart failure with preserved ejection fraction and diastolic dysfunction than male counterparts [52–54], who are more likely to develop heart failure with reduced ejection fraction and systolic dysfunction. This is likely a consequence of males typically developing macrovascular dysfunction and females developing microvascular dysfunction [55,56]. Postmenopausal females are more likely to develop both Takotsubo cardiomyopathy [57], which is often related to emotional stress resulting in increased levels of stress hormones and resultant cardiovascular dysfunction. In addition, females are more likely to develop spontaneous coronary artery dissection, which can impact both pre- and post-menopausal women, and can occur after stress (emotional and physical) and has similar clinical symptoms to MI [58]. Many of the therapeutic interventions for treating cardiovascular disease were originally tested and therapeutically optimized in males; however, as time passes, it is becoming evident that many of these treatment regimens may be suboptimal for females. For example, it was recently shown that females only needed 50% of the current recommended dose of β-blockers to effectively lower all-cause mortality in the setting of heart failure, which was also the case with Angiotensin converting enzyme inhibitors [59–61], and sex-dependent differences in the pharmacokinetics of calcium channel blockers have also been documented [62]. Sex differences are also apparent with respect to effectiveness and survival in the setting of non-pharmacological heart failure treatments, such as heart transplantation and implantable defibrillators [63]. These studies suggest that additional optimization is required for treating females with CVD.

Many animal models of cardiovascular disease also recapitulate the findings of protection in females. For example, in comparison with age-matched males, female mice exhibit lower mortality, diminished functional decline, increased capillary density, and reduced cardiac remodeling in response to MI [64,65]. After MI, females exhibit predilection to concentric hypertrophy and male hearts exhibit more eccentric hypertrophy, which is associated with changes in transcripts related to angiogenesis, extracellular matrix (ECM) remodeling, and the immune response [65]. The processes known to contribute to post-infarction remodeling and the potential underlying mechanisms that contribute to the increased resilience of the female heart in the setting of myocardial ischemia are outlined below.

4. Sexual dimorphism in processes known to contribute to post-infarction cardiac remodeling

MI results in a cascade of events involving changes in immune cell infiltration, which ultimately culminate in the development of a stable collagenous, fibrotic scar to compensate for the loss of cardiomyocytes. This fibrosis can then spread into non-infarcted regions to disrupt cardiac function further. Post-infarction remodeling of the myocardium also involves changes in surviving cardiomyocytes, which hypertrophy and change their metabolism; disconnection of cardiomyocyte-fibroblast connections resulting in arrhythmic activity; and changes in vascular rarefaction and angiogenesis.

4.1. Changes in immune cells between sexes

Heart disease is often associated with chronic inflammation which is an integral component of wound healing and scar formation after MI. Documented sex differences exist in both adaptive and innate immunity, with the general finding that females mount more significant immune responses after MI compared with males. In addition, it has been shown that immune cells differ in number between the sexes basally, with reduced leukocytes in female hearts [26], and differences have been observed in macrophage polarization, with male macrophages being more M1-like and female macrophages more M2-like in the setting of myocarditis [66]. Furthermore, male macrophages express more adrenergic receptors [67], which promote lipid accumulation, and female macrophages accumulate less cholesterol [68]. Aside from macrophages, neutrophil activation is differentially regulated between the sexes [69]. Compared with males, females appear somewhat protected from injury as they typically have reduced inflammatory cytokine production [70]. E2 also significantly modulates the immune response. For example, E2 reduced neutrophil infiltration and oxidative stress-induced cell injury in ischemia/reperfusion [71] through ERβ-dependent mechanisms [72]. In addition, E2 regulates monocyte chemoattractant protein-1 and reduces tumor necrosis factor alpha (TNFα), which is reversed by ovariectomy [73].

Studies analyzing the cardiac transcriptome of donor and postmortem hearts identified several transcripts located on the X-chromosome, involved in inflammation, that were sexually dimorphic, with females having a greater transcript expression of *Ccl4*, *Cx3cl1*, *IL32*, and *Nfkbia* [74]. However, this study did not link these transcriptomic changes to functional differences between sexes. Nevertheless, some studies have linked transcriptomic changes in immune cells to changes in cardiac function after MI. For example, Chen et al. [65] showed that, following MI, the female heart has higher capillary density and a reduction in fibrosis, which is consistent with increases in transcripts known to contribute to not only angiogenesis and extracellular matrix remodeling, but also to differences in transcripts that regulate the immune response, e.g., *Ccl4*, *Itgb1bp3*, *Cxcl19*, *Clec4n*, and *Rbp1*. Some immune cell transcripts upregulated in the female heart seem consistent between animal

and human cells, representing potential immune cell targets in the female heart that could be manipulated in future studies.

4.2. Changes in cardiac metabolism and mitochondrial function

Although few studies have examined sex differences in myocardial substrate utilization and metabolism, there are reports that provide some insight. For example, using positron emission tomography, it was shown that myocardial volume oxygen i.e., MVO_2 is higher and myocardial glucose utilization is lower in women compared with age-matched men [75]. The same group later examined these changes in the setting of nonischemic heart failure and showed that the women examined had higher myocardial blood flow, fatty acid uptake, and utilization, which was correlated with increased survival compared with men [76]. It has also been shown that sex-dependent differences exist in cardiac triglyceride metabolism. For example, in the setting of caloric restriction, male mouse hearts have greater triglyceride (TG) turnover and a smaller TG pool than females [77]. However, it has also been shown that male rat hearts have more significant TG accumulation than females, whereas females had reduced TG accumulation and accumulation of acylcarnitines [78]. Studies in teleost (i.e., trout) show that female hearts have higher lactate levels, lactate efflux, and lactate dehydrogenase activity and that male hearts have higher glycogen levels, citrate synthase activity and acyl-CoA dehydrogenase activity; these particular findings appear to be hormone-independent because the examined teleost was sexually immature [79]. The Delbridge lab corroborated these sex-dependent glycogen observations, which also showed that glycogen accumulation and glycophagy were more prominent in female hearts upon starvation [80]. Collectively, these studies suggest prominent sex-dependent differences in cardiac metabolism.

There also appears to be sex-dependent differences in the expression of several mitochondrial genes in the heart. For example, Vijay et al. [81], showed that the young female rat heart (i.e., eight weeks old) has significantly higher expression of fatty acid metabolism genes, including *Acaa2*, *Acads*, *Ech1*, *Hadhb*, *Hmgsc2*, *Mlycd*, and *Pcca*, and a substantial reduction in *Acsl4*. This expression profile changed with age (i.e., from 8 weeks to 78 weeks). In the same study, several mitochondrial complex subunits (e.g., complex I and IV complex subunits) were reduced in the female heart with advanced age compared with the aged male heart. Interestingly, the maternal heart exhibits significant reductions in many of these complex subunits [36], which may indicate remodeling of the mitochondrial subunit complexes during cardiac stress in the female heart. In addition, recent studies have shown that not only is there sexual dimorphism in the expression of mitochondrial genes and electron transport chain genes in the mouse heart, but these mitochondrial changes, specifically in *Acsl6*, contribute to diastolic dysfunction in the setting of heart failure with preserved ejection fraction (HFpEF) [82], which afflicts females disproportionately. Studies also suggest that mitochondria are protected from cardiac stress in the female heart. Female cardiomyocytes were shown to have improved mitochondrial respiration versus male cells in response to hydrogen peroxide and TNFα and preserved mitochondrial membrane potential in response to TNFα; interestingly, treatment of male cardiomyocytes with E2 recapitulated the protection shown in female cardiomyocytes [83]. Female mouse hearts also have higher mitochondrial mass compared with males, with male mitochondria having significantly rounder, more fragmented mitochondria; however, most of the functional analyses in this study were performed in brain mitochondria [84], so it is unclear whether these differences can be generalized to the cardiac mitochondrial pool.

Although metabolic remodeling plays a pivotal role in myocardial remodeling and has been documented in the heart following MI and in the development of heart failure, little is known about how post-MI heart metabolism differs with biological sex. Nevertheless, in the context of pressure overload, enhancing fatty acid oxidation through deletion of cardiac *Acacb* (*Acc2*) was shown to preserve cardiac function and improve cardiac energetics more in female mice than male mice [85]. Nevertheless, there remains little knowledge of whether such interventions are more beneficial in female mice in the context of ischemic insults. Regardless, these findings indicate significant sex-dependent differences in metabolism occurring in the context of cardiac stress. Additional studies are needed to establish the extent of sex-dependent changes in metabolism that occur following MI.

4.3. Sex differences in cardiac redox signaling

It is not surprising given the documented changes in metabolism observed between the sexes that cardiac redox signaling is also sexually dimorphic. Studies have linked the increased resilience of female myocardium to the increased S-nitrosylation of several proteins, including cyclophilin D and the F_1F_0-ATPase [86], in an I/R model. Interestingly, these proteins were not found to be modified in male hearts. This study also showed that female hearts had increased expression and phosphorylation of endothelial nitric oxide synthase (eNOS), increased nitric oxide (NO) production, and increased activity of S-nitrosoglutathione reductase in comparison to male hearts. In addition, female hearts were shown to have reduced cardiac protein carbonylation, reduced nitrotyrosine levels, and reduced abundance of advanced oxidation protein products in comparison to males [87]. This study also showed that female hearts had reduced glutathione levels but increased levels of glutathione disulfide ultimately resulting in a reduced redox index in the female heart. However, this latter study was performed in aged rats, it did not examine hormonal contributions, and was not in the setting of cardiac injury, so it is unclear whether this would also be the case in younger animals in response to cardiac stress. Increased resilience of the female heart to injury could also be the result of reduced production of reactive oxygen species, increased anti-oxidant capacity, and reduced lipid peroxidation. For example, the Murphy lab [88] has shown female hearts have reduced production of reactive oxygen species following I/R injury in comparison to males, which was associated with increased phosphorylation of α-ketoglutarate dehydrogenase and increased phosphorylation and activity of aldehyde dehydrogenase-2 in female hearts, contributing to sex-differences in mitochondrial ROS handling. These findings indicate significant sex-dependent differences in redox signaling in the heart, however, studies are needed to establish the extent and full contribution of these changes to increased female cardiac resilience following MI.

4.4. Cardiac fibrosis and remodeling of the ECM

Sex-specific differences are also apparent in the development and progression of cardiac fibrosis. In both animal models and humans, males are more prone to fibrosis in response to agonist stimulation and cardiac injury than females [64,89]. This sex-dependent difference persists in advanced age [90,91]. Recent evidence suggests that fibroblast activation, migration, and proliferation are differentially regulated between the sexes. Recently, Squiers et al. showed that fibroblasts are more abundant within the female heart [27]. Leinwand and colleagues showed that female cardiac fibroblasts are more proliferative in response to isoproterenol stimulation than male fibroblasts; however, the female fibroblasts were less activated due to lower expression of adrenergic receptors and protein kinase A activation [92]. It remains unclear whether additional factors and proteins contribute to the sex differences in fibroblast activation. In addition to changes in the activation of fibroblasts, the ECM produced by cardiac fibroblasts has been shown to be different between the sexes, which likely affords unique tensile properties to the matrix. For example, it has been shown that male hearts have greater collagen I content, whereas female hearts have a greater content of collagen III [90]. Other studies have shown that these collagens are lower in young female hearts and, upon aging, begin to increase due to diminishing E2 levels [93]. Notably, this pattern was reversed in the male heart. In addition, in response to fibroblast activation with Angiotensin II, sex-dependent gene expression was observed

in subpopulations of mouse fibroblasts, with four subpopulations (i.e., groups 2, 4, 5, and 6) exhibiting the most sexual dimorphism of the nine identified, which correlated with reduced fibrosis in the female heart [94]. Furthermore, critical regulators of ECM turnover have been shown to differ between the sexes, such as matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs). Circulating levels of MMP2 are reduced in females versus males with heart failure [95], which could be due to changes in E2 levels, given that E2 has been shown to contribute to reductions in MMP2 [96]. In addition, MMP3 is lower in female hearts in the setting of HFpEF [97]; however, it is unclear whether there are baseline differences in expression of MMPs between sexes. Nevertheless, there are documented sex differences in the activity and expression of TIMPs, with young females having low levels of TIMP1 and TIMP3 compared with males; this trend appears to reverse with aging [98]. In addition to changes in collagens between the sexes, changes in collagen stabilization and cross-linking enzymes have been observed. For example, lysyl oxidase expression is lower in young female mouse hearts compared with male hearts [99]. This study also identified sex-dependent changes in the expression of other essential ECM proteins (e.g., periostin, osteopontin, decorin); in negative regulators of collagen deposition (e.g., *Cthrc1*, *Ddr1*, and *Mrc2*); and in transforming growth factor-beta, transforming growth factor-beta receptor 1, and Smad2/3 signaling [99]. Collectively, these studies suggest that sex-dependent changes significantly impact the development of fibrosis in pathological conditions, such as MI, but also that fibroblast and ECM biology are regulated differently between the sexes, which could help to explain the reduced remodeling observed in the female heart with MI. What has yet to be established is whether the fibroblast secretome is sexually dimorphic, which could be an important factor shaping the differential responses to MI between the sexes.

Although E2 may protect the female heart from cardiac fibrosis through the inhibition of collagen production by estrogen receptor beta [100], inhibition of MMP2 [96], and through the reduced activation of fibroblasts [92,101,102], recent studies in ovariectomized mice suggest sex-dependent changes in fibrosis persist, suggesting non-estrogenic regulation of fibrosis. Of note, it has been documented up to 25% of genes escape X-linked inactivation [103]. Aguado et al. [104] recently identified the role of genes escaping X-linked inactivation as having an essential role in aortic fibrosis. However, contrary to findings suggesting reduced fibroblast activation in females, they found that *Bmx* and *Sts* escape X-linked inactivation in females and increase alpha-smooth muscle actin-mediated myofibroblast activation by activating RhoA/ROCK, endothelin-1, and plasminogen activator inhibitor-1 (PAI-1) signaling. This signaling mechanism was not observed in male hearts. Furthermore, TIMP1 has been shown to escape X-linked inactivation [105], which could afford some protection to the female heart. Male sex hormones could also play a role in regulating fibrosis. Testosterone has been shown to modulate fibroblast function in two studies [106,107]; however, discrepant findings persist and the precise mechanisms underlying sex differences in cardiac fibrosis remain poorly understood.

4.5. Changes in endothelial cell biology between sexes

As mentioned earlier, males are more at risk of developing atherosclerosis and resultant coronary artery disease than females. However, the risk in females increases with the onset of menopause, when females have higher arterial stiffness and an increased risk of plaque rupture. Literature suggests that sex-dependent differences are programmed in endothelial cells (ECs) from birth [108], and that male hearts possess more ECs than female hearts [26]. This sex difference in EC number remains in the setting of ovariectomy and castration, suggesting that this occurs independent of sex hormones [27]. There are also ten subpopulations of ECs that have been described [26] but it currently remains unclear whether changes in these subpopulations differ between the sexes under pathological conditions. Despite the reduction in EC number in the female heart, female hearts and endothelial cells [109] generate more NO, which could be due to higher expression and phosphorylation of eNOS. Increased eNOS and NO in female ECs was associated with increased migration, wound healing, and angiogenesis [109], which could contribute to preserved endothelial cell function in the female heart. Other sex differences in endothelial function include augmented viability, migration, and tubularization in female human umbilical vein ECs, which was associated with increased 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 levels and increased phosphorylation of focal adhesion kinase [110]. Recently, it was suggested that sex differences in endothelial function are the result of differences in mitochondrial Ca^{2+} handling, with female endothelial specific mitochondrial calcium uniporter KO mice having a larger reduction in acetylcholine-mediated vasodilation, which was associated with reduced mitochondrial Ca^{2+} uptake and was linked to changes in E2 [111]. In addition, several endothelial genes escape X-linked inactivation, such as *Shroom2* [112], which is important for EC migration and adhesion and could contribute to sex-dependent differences in EC function.

The endothelial secretome, which can alter vasodilatory and vasoconstriction processes, is also sexually dimorphic in nature. For example, male endothelial cells have been shown to produce more endothelin-1 compared with female endothelial cells [113], and PAI-1 and tissue type plasminogen activator—linked to increased cardiovascular disease risk—are higher in males compared with females [114]. It is not clear whether these sex-dependent differences in the EC secretome factor into the response to cardiac stress.

After MI, ECs can transition to mesenchymal-type cells in a process called endothelial-to-mesenchymal transition (EndoMT). Although sex differences have been described in EndoMT in the lung in the setting of pulmonary hypertension [115], it is unclear whether this is also the case in the infarcted heart. Nevertheless, it has been shown that, following MI, the EC transcriptome undergoes remodeling in a sex-dependent manner. In particular, *Fam5c*, which is an endothelial gene that participates in monocyte adhesion, is significantly upregulated in female hearts in response to MI, which may regulate the female immune response, resulting in increased protection from injury [116]. Endothelial Signal transducer and activator of transcription 3 (STAT3) has also been shown to contribute to sex differences in MI remodeling, as sex differences observed in wildtype mice were absent in the setting of EC-specific STAT3 KO [117]. This is interesting because both altered angiogenesis and reductions in cardiac STAT3 have been associated with female cardiovascular diseases observed during pregnancy [118–121] and could highlight a potential target in the female heart. Together, these findings suggest that significant sexual dimorphism exists in cardiac EC biology; however, additional studies are required to dissect the contribution of these changes to the increased cardiovascular resilience of the female heart to stress.

4.6. Changes in electrophysiological remodeling

In addition to changes in cardiac structure and function between the sexes, cardiac electrophysiology has been shown to differ between males and females. For example, women have higher heart rates compared with age-matched males [122]. Still, they also have longer QT interval [122], shorter sinus node recovery times [123], and increased parasympathetic activity [124], all of which impact electrical conduction in the heart and could contribute to the higher risk of LQT and *torsades de pointes* [125] in females. Not surprisingly, the expression and activity of ion channels contribute to cardiac repolarization differences between sexes. For example, female rabbit hearts have reduced K^+ currents (e.g., IKs), longer action potential duration, and changes in responsiveness to β-adrenergic stimulation [126]. These differences have been linked with E2 levels in females, as ovariectomy increased K^+ current. Of note, the increase in the action potential duration in female hearts also occurs in the failing heart; however, these changes were attributed to an increase in the L-type Ca^{2+} current (LTCC), more so than K^+ current [127].

Electrical remodeling is a significant component of remodeling that occurs in response to cardiac stress, such as ischemia. This involves disruption between individual cardiomyocytes and the connection between cardiomyocytes and other cell types in the heart. Gap junctions and connexins facilitate propagation of electrical activity between cardiomyocytes. Interestingly, sex-dependent expression of connexin-43 (Cx43) has been observed in the heart: female cardiomyocytes have higher Cx43 mRNA and protein expression compared with males, and, in response to agonist stimulation with phenylephrine, Cx43 expression was shown to be preserved in female cardiomyocytes compared with male cardiomyocytes [128]. These data suggest that female cardiomyocytes may somewhat preserve electrical coupling under periods of stress, which could contribute to increased resilience.

Sex-dependent differences in intracellular calcium, which shapes excitation-contraction coupling in the heart, also are apparent following injury. Compared with females, male mice and rats have increased calcium transient amplitude and duration. This difference in calcium responsiveness contributes to reduced cell shortening and changes in calcium spark frequency [129–132]. This is curious because female cardiomyocytes have increased expression of several calcium channels, such as LTCC and sodium/calcium exchanger (i.e., NCX1), versus males [133]. Some studies have also indicated increased Ca^{2+} current in female cardiomyocytes [127]. These differences are partly due to altered nuclear translocation of LTCC between the sexes and responses to E2 [134−136]. As mentioned above, responsiveness to β-adrenergic stimulation is sexually dimorphic, with female cardiomyocytes having a reduced response to isoproterenol compared with male cardiomyocytes [131,137,138], most likely a consequence of sex-dependent changes in PKA. Studies in the setting of ischemia/reperfusion (I/R) suggest increased S-nitrosylation of the LTCC in the female heart, which reduces Ca^{2+} entry and SR Ca^{2+} content [139] and could underlie better outcomes of the female heart after I/R, compared with the male heart.

4.7. Other factors that could contribute to sex-dependent changes in ventricular remodeling

In addition to the discussed biological processes known to change during MI, other factors are emerging that may play a role in shaping the remodeling response. One of these is the cardiomyocyte circadian clock, which dictates tolerance to I/R [140], with the onset of MI being time-of-day dependent [141]. Differences in the cardiomyocyte circadian clock were recently shown to contribute to sex differences in MI patients [142]. Because dysregulation of the cardiomyocyte circadian clock is associated with perturbations in cardiac metabolism as well as changes in cardiac function, fibrosis, and the immune response [143,144], sex-dependent differences in the clock could modify cardiac remodeling after insult or injury. However, because most studies on the cardiomyocyte circadian clock have not included female animals, additional work is required to determine the extent to which the clock contributes to sex-dependent cardiac responses to stress.

An additional mechanism that could be at play, alluded to earlier, is the impact of female-specific risk factors, such as hormonal fluctuations; hormonal-based contraceptive use; pregnancy and lactation; and pregnancy-associated cardiovascular disease. Although it is clear that female sex hormones have a significant impact on the biology of the female heart and explain some of sex differences, the exact mechanisms by which female-specific risk factors shape the response of the female heart to stress (e.g., MI) remain unclear. Inclusion of females in preclinical and clinical studies and considering these factors will provide a greater understanding of the female heart and its biological response to stress. Indeed, Lock et al. [145] and Chang et al. [146] recently proposed a framework that recommends female inclusion in preclinical and clinical studies and improved rigor when reporting on female subjects.

5. Conclusions

Although many sex differences have been reported to contribute to the development of cardiovascular disease and in the processes that modulate physiological and pathological cardiac remodeling, much more research is needed to address the current deficit in knowledge of the responses of the female heart to stress and aging. This deficit has been partially addressed by the implementation of sex as a biological variable by many funding agencies and publications. Still, additional rigor is required regarding factors relevant to sex, such as reporting the sex of cells used in *in vitro* experiments and accounting for hormonal, cycle status, and pregnancy history of experimental models. In addition, for clinical studies, steps should be taken to increase the recruitment of female participants through the modification of existing recruitment criteria, such as enforcing birth control. Including women in more studies will ultimately help the development of sex-specific treatment regimens and may help facilitate the incorporation of sex-specific factors into clinical practice. Furthermore, including females that have had prior pregnancies in both preclinical and clinical research will further assist in creating research populations that reflect population demographics.

Although sex hormones have an essential role in sexual dimorphism observed of the heart (e.g., in the regulation of cardiac structure, function, and metabolism), pathological remodeling processes such as fibrosis seem to occur independently of them, highlighting additional mechanisms that require interrogation. Notably, mechanisms remain unclear when hormones have been implicated in these scenarios, with conflicting results often reported. Furthermore, the impact of menopause should be considered further because it is unclear how menopause regulates underlying cardiovascular disease risk factors. Some studies suggest that the re-addition of hormones through hormone replacement therapy (HRT) to menopausal women can have both positive and adverse cardiovascular effects [147], which is likely in part the result of the timing of HRT. Evidence for this stem from experiments that underlie the basis of what is referred to as the “timing hypothesis”, in which studies showed that the cardiovascular benefit of HRT was observed when administered at the same time as ovariectomy rather than when given years after ovariectomy [148,149]. Since all sex hormones can have critical regulatory roles in shaping epigenetic changes, these should not be ruled out. Additional examination of genes that escape from X-linked inactivation or cases of X-linked gene reactivation should be considered in pathological settings. In addition, greater emphasis should be placed on establishing the impact of chromosomal changes in the response of the female heart to stress, in light of the recent findings regarding the four core genotypes model, and in terms of ischemic injury, where having two copies of the X chromosome resulted in enhanced injury in mice in the setting of I/R [150]. These studies suggest that chromosomal regulation of cardiac stress responses is a great deal more complex than we initially suspected.

Even with over seven hundred publications documenting sex-dependent changes in the failing heart, it’s clear that our knowledge on the topic is in its infancy, with newer studies suggesting that even the cellular composition of the heart is different between sexes. Thus, further study is required to interrogate not only the fundamental sex differences that exist in the hearts of males and females, but also to clarify the mechanisms that lead to improved outcomes in females in response to pathological stress.

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Declaration of competing interest

The author has no conflicts of interest and/or disclosures to declare. The author has no competing interests.

Data availability

Data will be made available on request.

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Abbreviations

Hadhb	3-ketoacyl-CoA thiolase
Acaa2	Acetyl-CoA Acyltransferase 2
ACC	Acetyl-CoA Carboxylase
Acsl	Acyl-CoA synthetase
ACE	Angiotensin-converting enzyme
CVD	Cardiovascular diseas
Ccl4	Chemokine ligands 4
Cxcl19	Chemokine ligand 19
Cthrc1	Collagen triple helix repeat containing 1
cx43	Connexin 4
Bmx	Cytoplasmic tyrosine protein kinase BMX
Clec4n	Dectin-2
DCM	Dilated Cardiomyopathy
Ddr1	DNA damage repair 1
Ech1	Enoyl-CoA Hydratase 1
E2	Estradiol
Erα	Estrogen receptor alpha
Erβ	Estrogen receptor beta
ECM	Extracellular matrix
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HRT	Hormone replacement therapy
HCM	Hypertrophic Cardiomyopathy
Hmgsc2	3-Hydroxy-3-methylglutaryl-CoA synthase
LTCC	L-type Ca^{2+} channel
Mlycd	Malonyl-CoA Decarboxylase
Mrc2	Mannose Receptor C-Type1
MMP	Matrix metalloproteinase
MI	Myocardial infarction
Itgb1bp3	Nicotinamide riboside kinase 2
PAI-1	Plasminogen Activator Inhibitor-1
Pcca	Propionyl-CoA Carboxylase subunit alpha
PKA	Protein kinase A
RhoA	Ras homolog family member A
Rbp1	Retinol binding protein 1
ROCK	Rho-associated, colied-coil-containing protein kinase
Acads	Short-chain acyl-CoA dehydrogenase
Smad2/3	SMAD family member 2/3
Sts	Steroid Sulfatase
TNFα	Tumor necrosis factor-alpha
TIMPs	Tissue inhibitor of matrix metalloproteinases
TG	Triglyceride

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