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Review

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Mechanisms and implications of sex differences in cardiac aging

Aykhan Yusifov[1], Kathleen C. Woulfe[2,3], Danielle R. Bruns[1,4]

[1]Kinesiology & Health, University of Wyoming, Laramie, WY 82071, USA.
[2]Division of Cardiology, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA.
[3]Division of Geriatric Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA.
[4]Wyoming WWAMI Medical Education, University of Wyoming, Laramie, WY 82071, USA.

Correspondence to: Dr. Danielle R. Bruns, Kinesiology & Health, and Wyoming WWAMI Medical Education, University of Wyoming, 1000 E. University Ave, Laramie, WY 82071, USA. E-mail: dbruns1@uwyo.edu

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Abstract

Aging promotes structural and functional remodeling of the heart, even in the absence of external factors. There is growing clinical and experimental evidence supporting the existence of sex-specific patterns of cardiac aging, and in some cases, these sex differences emerge early in life. Despite efforts to identify sex-specific differences in cardiac aging, understanding how these differences are established and regulated remains limited. In addition to contributing to sex differences in age-related heart disease, sex differences also appear to underlie differential responses to cardiac stress such as adrenergic activation. Identifying the underlying mechanisms of sex-specific differences may facilitate the characterization of underlying heart disease phenotypes, with the ultimate goal of utilizing sex-specific therapeutic approaches for cardiac disease. The purpose of this review is to discuss the mechanisms and implications of sex-specific cardiac aging, how these changes render the heart more susceptible to disease, and how we can target age- and sex-specific differences to advance therapies for both male and female patients.

Keywords: Cardiac, aging, sex differences, fibrosis, adrenergic desensitization

INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of death worldwide[1], with millions of individuals affected by CVD each year, making it a burdensome and costly public health problem. While aging impairs the cardiovascular system and is the dominant risk factor for CVD, CVD is not only a disease of the elderly. Rather, CVD is emerging in younger adults more often[2]. However, most of the research efforts on prevention and treatment of CVD ignore age and have focused on the development of interventions that target "traditional" CVD risk factors such as obesity, hypertension, and diabetes. While these risk factors are undoubtedly important and research efforts to understand them are critical to advance knowledge, more work is needed to understand the impact of age on the development of CVD, given the robust correlation between the development of disease and advanced age.

Aging is a complex biological process characterized by epigenetic alterations, genomic instability, cellular senescence, and mitochondrial dysfunction, amongst other cellular changes. Although there is substantial inter-individual variability in the aging process, it is clear that sex differences are present in aging. For example, women live longer than men, consistent with lower biological ages as assessed by molecular biomarkers[3]. Nonetheless, women are frailer and have worse health at the end of life[3-5]. Substantial clinical data demonstrates widespread sex differences with respect to cardiovascular structure and function. However, at present, there is relatively limited information on molecular mechanisms of sex-specific differences in cardiac aging and how sex-specific differences in the heart interact with the aging process. The reason for this lack of knowledge may be rooted in the long tradition of male-biased research[6] and some of the complications in including women and female animals due to hormonal fluctuations. Here we attempt to summarize what is known of cardiac aging and sex differences that contribute to distinct cardiac aging in men and women.

CARDIAC AGING IN MEN AND WOMEN

The aging heart

Aging leads to deterioration of cardiac structure and function in both men and women[7]. Although the mechanisms are not fully clear and are multifactorial, an important contribution to increased risk of CVD with advanced age lies in greater time for exposure to injurious stimuli, such as hypertension, metabolic stress, or ischemia, over the life course. Additionally, the heart loses its capacity for repair, meaning that with repeated injury, the cumulative burden of stress is elevated, increasing the risk of disease[8]. Thus, it makes sense that older patients would have greater impairment of cardiac reserves and elevated risk of disease.

With advanced age, the heart becomes hypertrophic, defined as abnormal enlargement, or thickening, of the heart muscle[9], largely because of an increase in ventricular myocyte size[10]. Elevated collagen levels and non-enzymatic cross-linking render collagen stiffer and also contribute to the ventricular thickness and tension[11,12]. This fibrotic process impairs ventricular function and reserve[13,14], resulting in impaired diastolic function[15]. Systolic function is typically preserved in healthy aging; however, systolic reserve is often diminished, resulting in a heart unable to increase contraction to meet metabolic demand. The mechanisms which underlie these changes in age-related cardiac function will be discussed in more detail below, with attention to the sex differences that underlie these changes in structure and function.

Sex differences in the aging heart and age-related cardiac disease

Sex-related differences in cardiac structure and function have been reported in several clinical studies[16-18]. Healthy women and men have different chamber dimensions and functions, even after indexing for body size. On average, females have a smaller left ventricular (LV) chamber with approximately 10% less LV mass

than males, even after indexing for body size by body surface area[19]. Since women have smaller LV chambers and accordingly lower stroke volumes, a higher resting heart rate maintains a similar cardiac output to men. Women also have higher systolic and diastolic LV stiffness than men at a given age, and these differences are more prominent with aging, where steeper increases in LV stiffness are seen in women compared to men[20]. Cardiac contractility is well preserved in women but declined in men after age 50[21]. These sex differences in function with age are also demonstrated pre-clinically, where animal studies show that systolic function declines with age in males but not in females[22,23]. Sex differences in the aged heart are also evident in cellular studies. Male cells contract more strongly and rapidly than female cells[24,25], driven likely both by differences in sarcomeric protein and calcium-handling function[24]. Even though the number of cardiomyocytes is similar between sexes at birth[26], female cardiac myocytes are less likely to undergo apoptotic cell death compared to male myocytes, resulting in an elevated risk for cardiac myocyte loss in male hearts[27]. Indeed, this observation is supported by a recent single-cell sequencing study which reported that human female hearts contain a significantly higher percentage of ventricular cardiomyocytes than male hearts at middle to early older age[28]. Together, current evidence clearly shows sex-specific differences in cardiac aging at the cellular, anatomical, and functional levels [Figure 1].

Sex differences in cardiac aging likely contribute to different age-related cardiac pathogenesis observed in clinical populations [Table 1]. Older men are more likely than older women to develop heart failure with reduced ejection fraction[29]. Women, on the other hand, are more likely to develop heart failure with preserved ejection fraction (HFpEF)[30,31], characterized by increased wall thickness and diastolic dysfunction with little or no reduction in ejection fraction. Men are more prone to ventricular arrhythmias[32,33]. Men are also at higher risk for coronary heart disease at younger ages[34], but women surpass men with age, and experience worse outcomes with myocardial ischemia, resulting in higher mortality and poorer quality of life[35-37]. In valvular heart disease, degenerative mitral regurgitation impacts a significant proportion of elderly women, particularly those with comorbidities[38]. While sex differences in cardiac physiology likely contribute to these clinical differences (discussed below), risk factors also vary between aged men and women, which contribute to different disease pathogenesis. For example, obesity, hypertension, and diabetes are highly prevalent in women with HFpEF, while the underlying cause of heart failure in men tends to be ischemia and coronary artery disease[39]. The contributions of these risk factors to the sex-specific development of cardiac aging are still being elucidated.

Sex differences in cardiac fibrosis in the aging heart

Both aging and diastolic dysfunction are characterized by elevated ventricular stiffness and deposition of extracellular matrix (ECM) proteins, thus cardiac fibrosis has been pursued by several research groups[40] as a mechanism of age-related heart disease. Indeed, fibrosis is associated with ventricular compliance and impaired passive filling of the LV[41,42]. Age-related alterations in cardiac ECM are sex-dependent, with aged male rat hearts more fibrotic than female hearts[43]. In human imaging-based studies, myocardial fibrosis is more pronounced in the aging male heart than the female heart[44,45].While the expression of collagen in human hearts did not differ between sexes, regulators of collagen metabolism differed between sexes[46]. Collagen types I and III were lower in young women than young men, but with age, the trend reversed, and women expressed higher collagen I and III compared to men[47]. Recently, it was reported that collagen I is the predominant type in the aged male heart, whereas collagen III was the main component in the aged female mouse heart[48]. Moreover, the authors correlated these sex-specific differences to sex-specific regional deposition of fibrosis, with males being more likely to undergo apoptosis and concomitant reactive interstitial fibrosis compared to females. In line with these studies, unpublished data from our lab suggests that the development of fibrosis with aging occurs in a temporally distinct manner in male versus female mice. We assessed collagen accumulation by Picro-Sirius red staining in the LV in mice from 4 distinct age groups: juvenile (4 weeks), adult (4-6 months), middle-aged (12 months), and aged (18 months) [Figure 2].

Table 1. Common age-related cardiac diseases that exhibit sex-specific differences

	Sex differences	Ref.
Heart failure	HFrEF more prevalent in men HFpEF more prevalent in women Women have more comorbidities, more likely to die with HFpEF	[29,30,31]
Ventricular arrhythmias	More prevalent in men	[32,33]
Ischemic heart disease	Higher risk for development of disease in men at younger age Worse outcomes and higher mortality for women following MI at older age	[29,35-37]
Valvular disease	Aortic regurgitation more prevalent in men Degenerative mitral valve disease more prevalent in elderly women (> 80 years)	[38]

HFrEF: Heart failure with reduced ejection fraction.

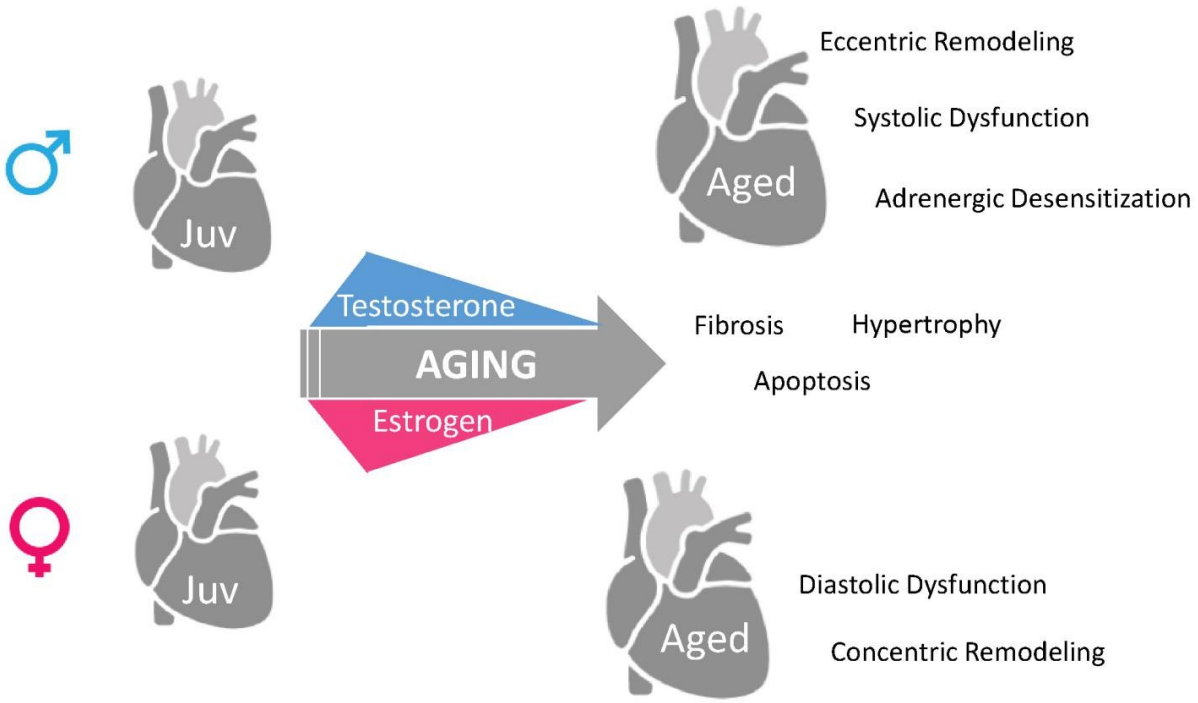

Figure 1. Sex-specific cardiac aging in male and female with respect to changes in major sex hormones testosterone and estrogen. While aging is characterized by ventricular hypertrophy, fibrosis, and changes in ventricular function, several mechanisms are more pronounced in the male heart compared to female. For example, the aged male heart demonstrates eccentric remodeling, systolic dysfunction, and lower adrenergic sensitivity as opposed to aged female heart, which demonstrates diastolic dysfunction and concentric remodeling. While some of these changes likely coincide with temporal changes in sex hormones, others are likely regulated by non-hormonal changes, or occur via different temporal patterns in the male and female heart.

Quantification of fibrosis showed that fibrotic content increases earlier in life for males, while this process was temporally delayed in females. When we analyzed the expression of key pro- and anti-fibrosis genes, we found that pro-fibrosis genes are upregulated in younger hearts of both sexes, and gradually decline with aging. Specifically, the expression of collagen I was higher in juvenile and adulthood and decreased in the aged female heart. In contrast, male hearts showed a decline in collagen I expression after juvenile-hood in mice[49]. On the other hand, the expression of anti-fibrotic genes gradually decreased with age, supporting the notion that accumulation of fibrous connective tissue in cardiac ECM is caused by slower removal of ECM components rather than due to increased deposition of fibrotic proteins into the matrix. These data suggest that reduced collagen degradation may be more important than increased *de novo* collagen synthesis in the pathogenesis of aging-associated fibrosis in a sex-specific manner. While activation of pro-fibrotic

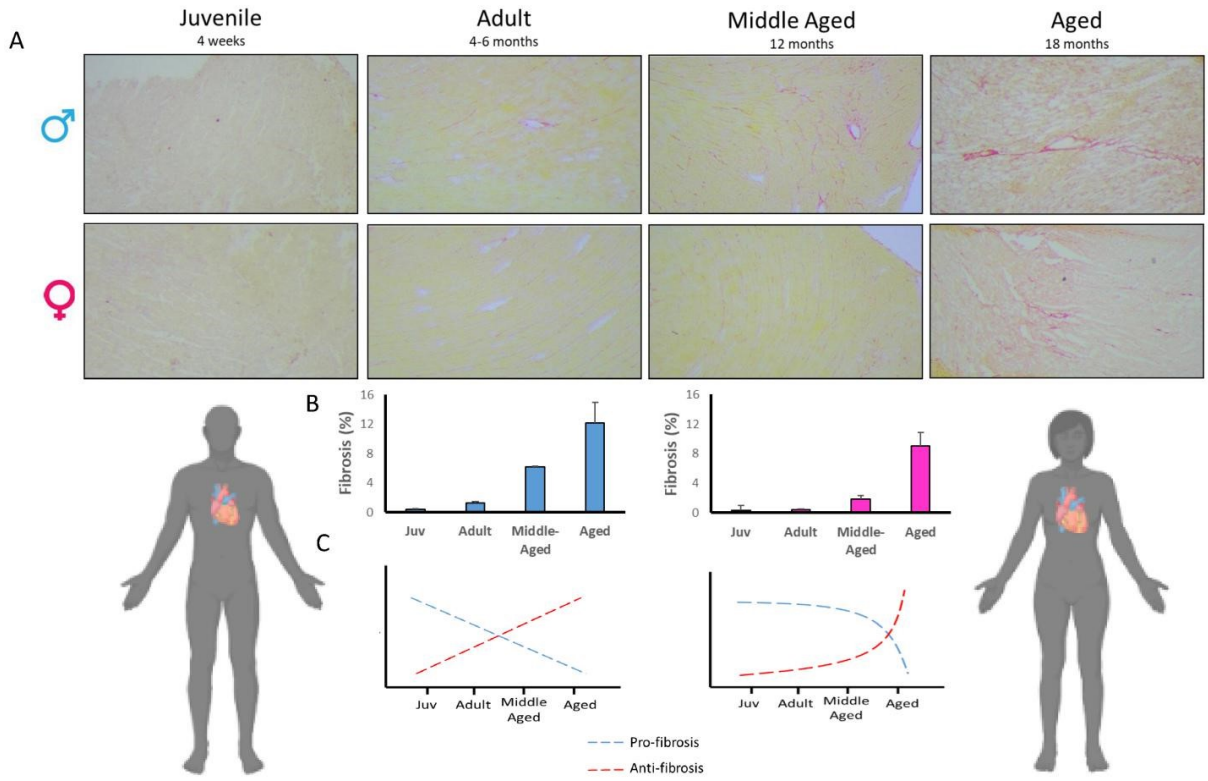

Figure 2. Fibrosis in the male and female heart across the life course. Collagen accumulation was assessed by picro-sirius red in LV in mice from 4 distinct age groups: juvenile (Juv; 4 weeks), adult (4-6 months), middle-aged (12 months), and aged (18 months) mice of both sexes. Quantification of fibrosis demonstrates that fibrotic content increases earlier in life for males, while females show relatively delayed fibrosis later in life. (A) Representative images; (B) quantification of fibrosis content in male and female samples. In male LV, fibrotic content was significantly higher in adult, while in female, fibrosis was not significantly elevated until middle age. *n* = 3/group; (C) expression of pro- and anti-fibrosis genes occurs in a sex-dependent manner with aging. Blue: male; pink/red: female.

genes is considered to be a primary pathway for the development of fibrosis, newly emerging data consider disruption of anti-fibrotic pathways as also essential in this process. It appears that the fibrotic process occurs via different temporal trajectories by sex. Future studies which utilize life-course approaches, and/or aim to understand the differences in animals of juvenile and middle ages will yield insight into the temporal nature of these sex-specific differences in cardiac aging. Understanding the mechanisms by which male and female hearts become fibrotic with advanced age is important for the identification of anti-fibrotic therapies- a large unmet clinical need.

Sex differences in aging at the cardiac myofilament

Even though whole cell contraction and relaxation are reliant on coordination from a myriad of regulatory processes in cardiomyocytes, the most basic contractile unit of the cardiomyocyte is the sarcomere. Sarcomeric protein interactions underlie contraction and relaxation, and thereby different protein isoforms or post-translational modifications on specific residues of sarcomeric proteins lead to significant differences in duration, rates, and intensity of contraction and relaxation. Simplistically, identifying key differences that occur at the level of the sarcomere between males and females as they age will provide the basis to understand larger, whole organ nuances and thereby provide therapeutic targets to modify aging-induced alterations.

It is clear that myofilament function is altered with age both in humans and animal models[50-58] but much like other cellular systems in aging, there is high heterogeneity in aging myofilament proteins. While conclusive functional changes are complicated by differences in animal models, age of the models, as well as differences in techniques, aging seems to modify passive stiffness, force generation, and calcium sensitivity, as well as prolong relaxation of the myofilament. However, it is clear that nuances in experimental design as well as, importantly, sex and health of the models warrant careful investigation and comparison. With this in mind, a study by Kane *et al.*[54,59] demonstrates the importance of incorporating a frailty assay or some assessment of whole-body aging as heterogeneity in biological aging clearly exists and likely confounds conclusions about myofilament modifications due to age. Moreover, this particular study makes it clear that the markers providing important information about aging may differ by sex since male animals had a significant correlation in their frailty scores to key modifications in their myofilament proteins, whereas female animals did not.

Of note, aging can induce altered myofilament function through either expression of different isoforms or through differential post-translational modifications. Nance *et al.*[60] reported that sarcomere lengthening is impaired in aged cardiomyocytes, which alters the length-dependent activation. One sarcomeric protein that regulates sarcomere length and contributes, in part, to the dynamics of sarcomeric lengthening is titin. Interestingly, a recent study determined that in male mice or humans, titin isoforms were not altered with age. However, phosphorylation at specific sites on titin were differentially modified in male mice[61]. Similarly, phosphorylation of serine 44 of cardiac troponin I is elevated, and contractile function decreased in aging rats[57]. Moreover, numerous reports suggest that myofilament proteins are differentially modified in males and females with age[54]. It is clear that sex hormones differentially impact myofilament function[62-69]. However, reports are conflicting with the overall effect of estradiol itself on myofilament function and modifications. Most notably, several studies demonstrate increased calcium sensitivity in response to acute loss of estradiol or low estradiol levels. However, chronic loss of estradiol leads to decreased myofilament calcium sensitivity. These contrasting reports suggest that changing estrogen levels at pivotal aging events such as perimenopause represent a different regulatory milieu than pre-menopause or even menopause. Decreased testosterone also has been shown to modify myofilament function, inducing prolonged relaxation and diastolic dysfunction in aged animals[62]. In line with this, orchiectomized male rats demonstrate decreased active force and slower cross-bridge cycling with higher expression of beta-myosin heavy chain and lower phosphorylation of key sarcomeric proteins[69]. However, an important consideration is that the majority of the sex hormone studies have been completed in adult or young animals and not in old animals. Therefore, while it is clear that estrogen and testosterone impact myofilament post-translational modifications, determining how age itself impacts myofilament along with hormonal changes is critical.

MECHANISMS OF SEX DIFFERENCES IN CARDIAC AGING

Sex differences in cardiac disease have long been attributed to estrogen, a hypothesis which largely stems from the loss of cardioprotection in women following menopause. While estrogen undoubtedly plays a significant role in the cardiac disease phenotype between men and women, the contribution of other sex hormones as well as non-hormonal mechanisms have emerged as critical regulators of cardiac disease and will be the focus of this section.

First, to help guide our discussion, we begin with a conceptual framework for understanding the basis of sex differences. This model was put forth by Arnold[70] to guide the study of sex-based differences in physiology and disease. This framework suggests that there are three causes of sex differences. First are the effects that are due to activation of gonadal steroid hormones, such that shortly after removing them, [i.e., by

Ovariectomy (OVX) and orchiectomy] the effects are attenuated. Several effects of gonadal steroid hormones persist following gonadectomy, and these are referred to as long-term organizational effects of hormones- i.e., the persistence of internal and external genitalia. Lastly, even following gonadectomy, there remain differences that cannot be explained due to steroidal hormones, but are rather due to the effects of sex chromosome genes acting outside the gonads. This framework leads to a relatively standard strategy for understanding sex-based differences and identifying sex-based factors-1) remove the gonads and quantify phenotypic changes. If they are present, identify which hormone (estrogens, testosterone, progesterone) drives the phenotype. If sex differences persist following gonadectomy, then organizational effects and non-hormonal effects should be studied. While this paradigm is simple and straightforward and certainly provides mechanistic insight, to date, the bulk of what is known about sex differences has focused predominantly on estrogen, with sex chromosome effects rarely investigated. We propose that a comprehensive analysis of sex hormone and chromosome-regulated mechanisms will yield a greater understanding of sex differences in cardiac aging and disease.

Sex hormone-mediated differences in cardiac aging

In premenopausal women, 17β-estradiol (E2) produced by the ovaries is the primary circulating estrogen. High concentrations of E2 act primarily as an endocrine factor on distal tissues. Serum estradiol concentrations are low in adolescence and increase at menarche. In adult women, estradiol fluctuates with the menstrual cycle, ranging from 100 pg/mL in the follicular phase to about 600 pg/mL at ovulation. Estradiol is high during pregnancy, and then after menopause, concentrations fall to similar values or lower to those in age-matched men (5 to 20 pg/mL). Following menopause and in men, extragonadal sources are responsible for the low levels of E2 production, largely acting in paracrine roles. While aging female rodents do not undergo true menopause, they do become reproductively incompetent or senescent with advanced age, a state referred to as estropause. Estropause is characterized by persistently lower estrogen, varying length of the estrous cycle, with eventual cessation of cyclicity around 12-14 months of age[71]. Ovariectomy of aged animals supports the loss of estrogen with aging, as animals do not undergo changes in metabolic function with ovarian estrogen removal, nor do significant changes in cardiac phenotype occur with removal of the ovaries[72]. However, OVX and aging are not synonymous. While they both are characterized by loss of ovarian estrogen, as discussed above, the aged heart is phenotypically distinct from the adult- both in the basal as well as the stressed state. Thus, we propose that future work dissect the impact of estrogen from aging on cardiac function using approaches which uncouple biological age from ovarian estrogen status.

Most data regarding estrogen signaling refers to E2. E2 is inversely associated with cardiovascular disease events in postmenopausal women, with women maintaining high estrogen levels having lower heart disease risk[73]. A wealth of data has demonstrated beneficial effects of E2 treatment on the heart, including reduced fibrosis, attenuated oxidative stress, improved mitochondrial function, and attenuation of cardiac hypertrophy[74]. Estrogenic effects in the heart are due to signaling through estrogen receptors α and β (ERα and ERβ) as well as G-protein-coupled ER (GPER). It is well-accepted that the myocardium is responsive to circulating androgens and estrogens, due to the expression of ERα and ERβ in the myocardium, likely in multiple cell types including myocytes and cardiac fibroblasts[75]. E2 binds ER, the complex internalizes, translocates to the nucleus, and activates transcription of estrogen-responsive genes. In addition to the protection afforded by E2 *in vitro* and in pre-clinical models of cardiac disease, the beneficial role of E2 is also supported by studies that show depletion of ovarian estrogen by OVX reverses the protective effects of E2[76]. These gain and loss of E2 studies, along with the epidemiology data showing clear loss of cardiovascular disease protection with menopause, led to the early hypothesis that restoration of E2 with aging would reduce heart disease morbidity and mortality. However, early studies to give back E2 to the aged heart were not successful[77]. Aging has been suggested to diminish the ability of estrogen to be

protective, and a "timing" hypothesis for estrogen therapy has emerged[78]. In aged spontaneously hypertensive rats, E2 delivery did not attenuate hypertrophy or molecular signatures of the failing heart, such as myosin heavy chain expression[78]. The authors suggested that this was in part due to not only reduced E2 synthesis but also impaired estrogen metabolism, given differences in expression of 17β-HSD, which catalyzes the reduction of weak estrogens into potent estrogens like E2. Indeed, estrogen metabolites (2-hydroxyestradiol and 2-methoxyestradiol) are also emerging as regulators of cardiac function, likely through some similar mechanisms as E2. Age-related changes in estrogen receptor and GPER expression have also been studied as a mechanism to explain the loss of cardioprotection with age. While cardiac GPER expression appears to increase with age in mice of both sexes, cardiac ERα decreases with age in females but remains unchanged in males[79]. Together, while it is clear that E2 is cardioprotective and loss of E2 occurs with advanced age, the mechanisms by which E2 protects the heart, how this protection declines with age, and effective therapeutic strategies for interventions against age-related declines have yet to be fully elucidated.

Testosterone decreases with advanced age, not only in men[80], but also in women[81]. Age-dependent reductions in testosterone are also evident in aged male rodents[82]. Considerable evidence has emerged that low testosterone may therefore also contribute to increased risk for heart disease with aging[73,83,84]. The link between testosterone and heart disease is particularly strong for diseases of impaired contractility such as heart failure. The biological effects of testosterone occur through androgen receptors expressed in the heart, at least in cardiac myocytes[85]. Binding of androgens causes transcriptional regulation of androgen-responsive genes. However, similar to estrogen, non-genomic actions of testosterone have also been described.

Mechanistic understanding of androgens on cardiac function comes from studies of gonadectomy. In young mice, bilateral orchiectomy attenuates contractile function[86]. Long-term withdrawal of testosterone slows relaxation[62]. On the other hand, epidemiological studies of anabolic steroid users indicate that high levels of exogenous testosterone negatively impact the heart[87]. The long-term effects of modest or physiological levels of testosterone on the heart remain unsettled, as evidenced by the recent initiation of a clinical trial to determine the effects of long-term testosterone treatment on cardiovascular outcomes[88]. The impact of gonadectomy in the aging heart is not clear but likely differs from those in the young, given that gonadectomy in aged mice did not change testosterone or measures of muscle mass - findings contrary to those reported in younger male mice[89].

In addition to direct androgen effects, testosterone also impacts cardiac function due to the fact that estrogen biosynthesis is dependent on testosterone availability. Testosterone can be converted to E2 by aromatase, expressed in non-gonadal tissue. While the expression of aromatase in the heart is contentious[79,90], even low expression may meaningfully contribute to E2 synthesis in a setting of low systemic concentrations. E2 produced by aromatase has been speculated to act locally in a paracrine or autocrine manner, rather than as a hormone as when gonadal E2 synthesis is intact. While the cellular location of aromatase expression has not been fully described, there is some evidence that it is predominantly expressed in the coronary vasculature, with lower expression in cardiac myocytes[91]. To date, the expression of aromatase in cardiac fibroblasts is not known. Cardiac localized aromatase likely controls the balance between testosterone and estrogen, permitting sex steroid regulation of cardiac function. In support of this hypothesis, deletion of aromatase profoundly alters the cardiac stress response[90]. Understanding the contribution of aromatase to E2 synthesis in the postmenopausal and aging heart is an area ripe for future research.

As alluded to above, dissection of aging from loss of gonadal hormones with advanced age is not yet clear. That is- are age-related changes in cardiac function due to natural aging, due to age-related declines in estrogen, or both? Clinical studies of women in different phases of the menstrual cycle as well as peri-, menopausal, and postmenopausal women, suggest that while sex hormones (especially E2 and FSH) regulate arterial stiffness, the effects are largely driven by age[92]. In support of this hypothesis, OVX did not cause independently cause cardiac remodeling and dysfunction in rats, but rather aging resulted in diastolic dysfunction and mild systolic impairment[93]. Together, these findings suggest that associations between hormones and cardiovascular function are likely different at different ages or reproductive stages. These types of studies should be investigated by removal of gonads in aged mice and quantifying cardiac function in aged animals.

Sex chromosome mediated mechanisms of cardiac aging

We recently reported significant differences in the cardiac transcriptome that are apparent before the onset of major sex hormones and sexual maturity, as well as in the hearts of reproductively incompetent aged females[49]. The cardiac proteome also appears to develop differently between males and females, independent of sex hormones[94], given the robust differences in proteomics in mice at embryonic day E9.5, with gonadal development occurring at day E11. When sex hormones cannot explain sex differences, either due to the persistence of sex differences post removal of gonads, it is logical to conclude that non-hormone mediated mechanisms must be contributing.

Clinical genomics studies suggest that age-related cardiac disease develops in conjunction with sex chromosomes. Polysomy of the Y chromosome is associated with elevated CVD mortality due to atherogenic lipid profiles[95]. In addition, women with monosomy X (Turner Syndrome) also have an elevated risk for coronary artery disease[96]. However, in humans, sex chromosome aneuploidy conditions (XO and XXY) are also associated with aberrant hormonal levels, making it difficult to separate chromosomal effects. To circumvent this problem, several mouse models have been generated which permit the dissection of sex chromosome differences from gonadal hormones. For the purposes of this review, we will discuss two of such models, though we refer the reader to reviews on sex hormones and chromosomes in CVD for more details[97]. To dissect the contributions of sex hormones from chromosomes, mice have been generated in which sex chromosomes are separate from gonadal hormones. In the case of the four core genotype (FCG) mouse, two separate mutations delete the testis-determining gene (Sry) from the Y chromosome or insert it into an autosome. This results in an XX female mouse, an XY- mouse which develops ovaries and subsequently ovarian hormones, as well as XY-Sry and XXSry transgenic mice that are gonadal males. If XX and XY mice differ despite similar hormone levels, then sex chromosomes are likely responsible for the phenotype. The XY* mouse is the other common model, where XY* mice possess the Y* chromosome, which has an aberrant pseudoautosomal region with permits crossing over with X chromosome during meiosis. This produces abnormal recombination of X and Y. Mating XY* males to XX females produces progeny that are gonadal males or females, each with one *vs.* two X chromosomes.

Given the relatively recent emergence of these models, cardiac and certainly aging studies utilizing them are sparse. In the FCG mouse model, XX mice, compared to XY mice, had more beneficial lipid profile in the form of elevated high-density lipoprotein[98]. The magnitude of hypertension induced by angiotensin II is greater in gonadectomized XX mice compared to XY[99]. In the XY* mouse model, at baseline at two months of age, all mice had a similar cardiac function, but X mice have higher vulnerability to I/R injury compared with XY* mice, due to the number of X chromosomes rather than the absence of the Y chromosome[100].

Collectively, it is clear that neither hormones nor chromosomes alone are responsible for sex differences in cardiac disease and aging. Rather, the two mechanisms likely work separately as well as through overlapping or intersecting mechanisms to regulate cell and organ-level physiological and molecular differences. A recent publication using the FCG model quantified the cardiac proteins under the regulation of hormones (proteins that segregated with ovaries and testes) versus those that were chromosomally controlled (segregated with chromosomes). While the authors identified 519 under hormonal control and 159 proteins under chromosomal control, they also identified a subset that involved a combination of chromosome and hormonal control[94]. They speculated that these genes are regulated by both mechanisms or, could be driven by chromosomes but opposed by hormones. In the latter interpretation, male and female disease phenotypes would be similar in presentation, but the mechanism of development would vary by sex. In this case, treatment of the disease could also vary between males and females. With respect to aging, as briefly discussed above, dissecting chromosomes and age-related changes in hormones is necessary to fully elucidate sex differences in the heart. In a series of experiments aimed at understanding how gonadal hormones and chromosomes influence hypertension, adverse sex chromosome effects which contributed to hypertension were exacerbated by the removal of gonadal estrogen by OVX. That is, the interaction between the X chromosome and estrogen could contribute to hypertension in postmenopausal women[99]. To date, these types of investigations are incredibly limited and warranted to dissect the mechanisms of cardiac aging in male and female hearts.

SEX DIFFERENCES IN THE CARDIAC STRESS RESPONSE VIA THE ADRENERGIC CASCADE

While sex differences in healthy aging are important, as discussed above, so are sex differences in the aged heart response to stress and/or ability to tolerate stress. The aging heart is well-characterized by a diminished stress response, resulting in elevated morbidity and mortality compared to younger animals. In young models, the female advantage is also well-characterized, with young females being at less risk of developing heart disease compared to males of similar age[101]. The female advantage declines with advanced age, as evidenced by a steeper rate of heart disease risk increase in women older than 50 compared to men[102]. However, given the assumption that sex differences disappear with estro/menopause, differences in the aged male and female heart to cardiac stress have been sparsely studied. These studies are also complicated by the diminished ability of aged cohorts to survive cardiac insult, further contributing to a lack of sex difference studies in the aging heart.

In response to cardiac stress, compensatory mechanisms are engaged in an effort to maintain cardiac function[103]. One of these major compensatory mechanisms is the activation of neuro-hormonal system, largely mediated by the stimulation of adrenergic receptors (AR) by catecholamines[104,105]. Activation of AR and downstream signaling increases calcium sensitivity and phosphorylates myofilament contractile proteins, shifting calcium affinity and contractile dynamics, in an effort to increase cardiac work. The human heart expresses two broad classes of adrenergic receptors, the α-adrenergic and the β-adrenergic families. Each of these families can be further subdivided into subclasses, but since the β-adrenergic receptor (β-AR) cascade has been the focus of active research in the context of cardiac function, we will focus on sex differences in the β-AR and changes in this cascade with advanced age.

β-AR are members of the G protein-coupled receptor (GPCR) superfamily of receptors. There are three major subtypes of β-ARs identified in the human heart, β1-, β2-, and β3-AR[106-108], and of these, β1-AR and β 2-AR have earned the greatest interest due to their role in myocardial contraction. In brief, activation of the cascade starts with the binding of β-AR agonist, which causes a conformational change in the receptor. A primary effect of the β-AR is stimulation of adenylyl cyclases, multiple subtypes of which are expressed in

human cardiac tissues. Adenylyl cyclases catalyze the conversion of ATP to the second messenger cAMP, which in turn binds to the regulatory subunits of protein kinase A (PKA). PKA phosphorylates serine and threonine residues on a number of proteins, thereby affecting a spectrum of cellular processes ranging from contractility to global gene expression patterns. Important PKA targets that acutely modulate myocardial contractility are β-ARs, L-type Ca^{2+} channels, the sarcoplasmic reticular Ca^{2+}ATPase inhibitory protein, phospholamban, and troponin I (TnI)[109]. Once the incoming signal is transduced, termination of the signal is accomplished to balance activation and deactivation. β-ARs deactivation is mainly accomplished through the actions of GPCR kinases (GRKs)[110]. GRK recruits β-arrestins, which uncouple the receptor from G-proteins and promote internalization and down-regulation of the receptor[110].

Adrenergic regulation of cardiac function with aging

Age-related declines in the responsiveness of adrenergic activation are well-established[111]. Decline in sensitivity to catecholamine stimulation has been attributed to high levels of circulating catecholamines, which lead to downregulation of β-adrenergic receptor and pathway activation[105,112,113]. Diminished β-adrenergic receptor sensitivity is called "β-adrenergic desensitization" and is characterized by altered ventricular inotropic reserve and exercise intolerance[111,114]. The underlying mechanisms have not been fully elucidated, but evidence suggests that fewer β-adrenergic receptors, other components of the β-adrenergic signaling pathway, or a combination play a role[111,112,114,115]. In addition to adrenergic desensitization, aging also influences the cardiac response to adrenergic-mediated therapeutics. Stimulation of AR with receptor agonists has been reported to have a deleterious effect on cardiac function in patients over the age of 65[116]. Although to date, no large clinical trial has specifically set out to examine β-blockade in older patients, a small uncontrolled observational study with patients with a mean age of 78 noted twice the rate of withdrawal and no improvements in symptoms of chronic heart failure[117] compared to younger patients. Given the strong association of advanced age with heart disease risk, understanding the impact of age on adrenergic therapeutics such as β-blockade is warranted.

Sex differences in the cardiac adrenergic cascade

In addition to differences with age, sex differences also exist in cardiac function in response to AR stimulation. Female patients maintain cardiac output through changes in heart rate[118], while male hearts tend to utilize changes in Frank-Starling mechanisms to increase cardiac output. Animal studies also reveal sex differences in AR stimulation and cardiac performance. Data from our group in a model of isoproterenol-mediated adrenergic activation support this observation, with a significant increase in cardiac output in adult females, driven by higher heart rate alongside unchanged stroke volume. Isoproterenol also resulted in sex-specific changes in cardiac structure, function, and gene expression across age and sex[119], sex differences which were differentially changed with age. Previous reports of catecholamine stimulation in humans demonstrated that adrenaline infusion in young (average age 30) and older (average age 60) adults caused similar increases in heart rate, but larger increases in stroke volume and ejection fraction in younger compared to older subjects[120]. Additionally, young males demonstrated a greater increase in heart rate than young females in response to adrenergic receptor stimulation. Together, these data suggest that with age, males demonstrate exacerbated decline in adrenergic-mediated function.

Despite the well-established observation that declines in adrenergic activity occur with aging and disease and that β-ARs are key pharmaceutical targets, only a few studies have investigated mechanistic sex differences, and the results of these studies have been inconsistent[121-123]. Gonadal hormones influence the response to the β-blockers in animal models. In a rat study, β-blocker treatment was effective only in males, but not in gondally intact females[124,125]. When it comes to the comprehensive assessment of the adrenergic cascade, the majority of the studies investigating sex differences have assessed the contractile response[121,126] with a smaller number of studies aimed at understanding calcium handling[127,128]. For instance, when the

expression and abundance of calcium handling proteins are compared between sexes, female rats showed significantly higher levels of these proteins[129]. We recently reported that β_1-AR and β_2-AR expression and several downstream AR targets were altered in a sex- dependent manner in response to isoproterenol. While expression of β2-AR was several fold increased in aged male hearts in response to AR stimulation, it remained unchanged in the aged female[119]. Sex differences have been reported in the activity of adenylyl cyclases and cAMP production[126,127], as well as the activity of phosphodiesterases in cAMP breakdown[130]. These studies report that female myocytes have lower levels of basal cAMP. If basal cAMP is lower in females, this would be expected to cause less PKA activation. Indeed, downstream measurements of PKA activity show sex differences, in such that stimulation of β-AR increased Ca^{2+} currents, Ca^{2+} transients and contraction in myocytes from females in comparison to males[130]. Testosterone inhibits phosphodiesterase activity in the rat heart[131]. The inhibition of phosphodiesterase can potentially explain higher levels of basal cAMP in male hearts compared to females. While these previous findings clearly demonstrate that sex differences exist in the activation of the adrenergic cascade, and that these differences change with age, it is clear that more work is necessary to investigate mechanistic differences in the AR cascade. Given the widespread use of pharmacological agents which target the adrenergic cascade, studies utilizing sex and age as biological variables are needed.

CONCLUSIONS

Sex differences underlie many facets of cardiac aging, including prevalence, severity and manifestation and susceptibility to a variety of heart diseases. However, despite the observation that men and women age differently (as typified by premenopausal cardioprotection), the mechanisms of sex-specific cardiac aging still remain unclear. Understanding the contributions of sex and age, as well as their complex interplay in the context of cardiac health, represents a fundamental step toward sex-and age-specific medicine and the development of more effective options to prevent and treat heart disease for both male and female patients.

DECLARATIONS

Authors' contributions

Conceived and drafted the manuscript: Yusifov A, Woulfe KC, Bruns DR

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

REFERENCES

1. Roth GA, Mensah GA, Johnson CO, et al; GBD-NHLBI-JACC. Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the GBD 2019 study. *J Am Coll Cardiol* 2020;76:2982-3021. DOI PubMed PMC
2. Prevention C for DC and (2018) underlying cause of death, 1999-2018. In: CDC WONDER Online Database. Available from: https://wonder.cdc.gov/ucd-icd10.html [Last accessed on 15 Mar 2022].
3. Jylhävä J, Pedersen NL, Hägg S. Biological age predictors. *EBioMedicine* 2017;21:29-36. DOI PubMed PMC
4. Austad SN, Fischer KE. Sex differences in lifespan. *Cell Metab* 2016;23:1022-33. DOI PubMed PMC
5. Gordon EH, Peel NM, Samanta M, Theou O, Howlett SE, Hubbard RE. Sex differences in frailty: a systematic review and meta-analysis. *Exp Gerontol* 2017;89:30-40. DOI PubMed
6. Holdcroft A. Gender bias in research: how does it affect evidence based medicine? *J R Soc Med* 2007;100:2-3. DOI PubMed PMC
7. Fleg JL, Strait J. Age-associated changes in cardiovascular structure and function: a fertile milieu for future disease. *Heart Fail Rev* 2012;17:545-54. DOI PubMed PMC
8. Angert D, Berretta RM, Kubo H, et al. Repair of the injured adult heart involves new myocytes potentially derived from resident cardiac stem cells. *Circ Res* 2011;108:1226-37. DOI PubMed PMC
9. Samak M, Fatullayev J, Sabashnikov A, et al. Cardiac hypertrophy: an introduction to molecular and cellular basis. *Med Sci Monit Basic Res* 2016;22:75-9. DOI PubMed PMC
10. Vasan RS, Levy D. Defining diastolic heart failure: a call for standardized diagnostic criteria. *Circulation* 2000;101:2118-21. DOI PubMed
11. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part II: the aging heart in health: links to heart disease. *Circulation* 2003;107:346-54. DOI PubMed
12. Scholz DG, Kitzman DW, Hagen PT, Ilstrup DM, Edwards WD. Age-related changes in normal human hearts during the first 10 decades of life. Part I (Growth): a quantitative anatomic study of 200 specimens from subjects from birth to 19 years old. *Mayo Clinic Proceedings* 1988;63:126-36. DOI PubMed
13. Schulman SP, Lakatta EG, Fleg JL, Lakatta L, Becker LC, Gerstenblith G. Age-related decline in left ventricular filling at rest and exercise. *Am J Physiol* 1992;263:H1932-8. DOI PubMed
14. Benjamin EJ, Levy D, Anderson KM, et al. Determinants of doppler indexes of left ventricular diastolic function in normal subjects (the Framingham heart study). *Am J Cardiol* 1992;70:508-15. DOI PubMed
15. Peverill RE, Chou B, Donelan L, Mottram PM, Gelman JS. Possible mechanisms underlying aging-related changes in early diastolic filling and long axis motion-left ventricular length and blood pressure. *PLoS One* 2016;11:e0158302. DOI PubMed PMC
16. Claessens TE, Rietzschel ER, De Buyzere ML, et al. Noninvasive assessment of left ventricular and myocardial contractility in middle-aged men and women: disparate evolution above the age of 50? *Am J Physiol Heart Circ Physiol* 2007;292:H856-65. DOI PubMed
17. Kane GC, Karon BL, Mahoney DW, et al. Progression of left ventricular diastolic dysfunction and risk of heart failure. *JAMA* 2011;306:856-63. DOI PubMed PMC
18. Kerkhof PLM, Peace RA, Heyndrickx GR, Meijboom LJ, Sprengers RW, Handly N. Heart function analysis in cardiac patients with focus on sex-specific aspects. In: Kerkhof PLM, Miller VM, editors. Sex-specific analysis of cardiovascular function. Cham: Springer International Publishing; 2018. p. 361-77. DOI PubMed
19. Fuchs A, Mejdahl MR, Kühl JT, et al. Normal values of left ventricular mass and cardiac chamber volumes assessed by 320-detector computed tomography angiography in the Copenhagen General Population Study. *Eur Heart J Cardiovasc Imaging* 2016;17:1009-17. DOI PubMed
20. Redfield MM, Jacobsen SJ, Borlaug BA, Rodeheffer RJ, Kass DA. Age- and gender-related ventricular-vascular stiffening: a community-based study. *Circulation* 2005;112:2254-62. DOI PubMed
21. Burstein B, Nattel S. Atrial fibrosis: mechanisms and clinical relevance in atrial fibrillation. *J Am Coll Cardiol* 2008;51:802-9. DOI PubMed
22. Medrano G, Hermosillo-Rodriguez J, Pham T, et al. Left atrial volume and pulmonary artery diameter are noninvasive measures of age-related diastolic dysfunction in mice. *J Gerontol A Biol Sci Med Sci* 2016;71:1141-50. DOI PubMed PMC
23. Koch SE, Haworth KJ, Robbins N, et al. Age- and gender-related changes in ventricular performance in wild-type FVB/N mice as evaluated by conventional and vector velocity echocardiography imaging: a retrospective study. *Ultrasound Med Biol* 2013;39:2034-43. DOI PubMed PMC
24. Parks RJ, Howlett SE. Sex differences in mechanisms of cardiac excitation-contraction coupling. *Pflugers Arch* 2013;465:747-63. DOI PubMed PMC
25. Trexler CL, Odell AT, Jeong MY, Dowell RD, Leinwand LA. Transcriptome and functional profile of cardiac myocytes is influenced by biological sex. *Circ Cardiovasc Genet* 2017;10:e001770. DOI PubMed PMC
26. Senyo SE, Lee RT, Kühn B. Cardiac regeneration based on mechanisms of cardiomyocyte proliferation and differentiation. *Stem Cell Res* 2014;13:532-41. DOI PubMed PMC
27. Biondi-Zoccai GG, Agostoni P, Sangiorgi GM, et al; Real-world Eluting-stent Comparative Italian retrosPective Evaluation Study Investigators. Incidence, predictors, and outcomes of coronary dissections left untreated after drug-eluting stent implantation. *Eur Heart J* 2006;27:540-6. DOI PubMed
28. Litviňuková M, Talavera-López C, Maatz H, et al. Cells of the adult human heart. *Nature* 2020;588:466-72. DOI PubMed PMC
29. Dunlay SM, Roger VL, Weston SA, Jiang R, Redfield MM. Longitudinal changes in ejection fraction in heart failure patients with

preserved and reduced ejection fraction. *Circ Heart Fail* 2012;5:720-6. DOI PubMed PMC

30. Aurigemma GP, Gottdiener JS, Shemanski L, Gardin J, Kitzman D. Predictive value of systolic and diastolic function for incident congestive heart failure in the elderly: the cardiovascular health study. *J Am Coll Cardiol* 2001;37:1042-8. DOI PubMed
31. Kitzman DW, Gardin JM, Gottdiener JS, et al. Importance of heart failure with preserved systolic function in patients ≥ 65 years of age. *J Am Coll Cardiol* 2001;87:413-9. DOI
32. Lampert R, McPherson CA, Clancy JF, Caulin-Glaser TL, Rosenfeld LE, Batsford WP. Gender differences in ventricular arrhythmia recurrence in patients with coronary artery disease and implantable cardioverter-defibrillators. *J Am Coll Cardiol* 2004;43:2293-9. DOI PubMed
33. Rho RW, Patton KK, Poole JE, et al. Important differences in mode of death between men and women with heart failure who would qualify for a primary prevention implantable cardioverter-defibrillator. *Circulation* 2012;126:2402-7. DOI PubMed
34. Benjamin EJ, Blaha MJ, Chiuve SE, et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics-2017 update: a report from the American Heart Association. *Circulation* 2017;135:e146-603. DOI PubMed PMC
35. Millett ERC, Peters SAE, Woodward M. Sex differences in risk factors for myocardial infarction: cohort study of UK Biobank participants. *BMJ* 2018;363:k4247. DOI PubMed PMC
36. Bugiardini R, Cenko E. Sex differences in myocardial infarction deaths. *Lancet* 2020;396:72-3. DOI PubMed
37. Aggarwal NR, Patel HN, Mehta LS, et al. Sex differences in ischemic heart disease: advances, obstacles, and next steps. *Circ Cardiovasc Qual Outcomes* 2018;11:e004437. DOI PubMed
38. DesJardin JT, Chikwe J, Hahn RT, Hung JW, Delling FN. Sex differences and similarities in valvular heart disease. *Circ Res* 2022;130:455-73. DOI PubMed PMC
39. Greiten LE, Holditch SJ, Arunachalam SP, Miller VM. Should there be sex-specific criteria for the diagnosis and treatment of heart failure? *J Cardiovasc Transl Res* 2014;7:139-55. DOI PubMed PMC
40. Horn MA, Trafford AW. Aging and the cardiac collagen matrix: novel mediators of fibrotic remodelling. *J Mol Cell Cardiol* 2016;93:175-85. DOI PubMed PMC
41. van Heerebeek L, Hamdani N, Falcão-Pires I, et al. Low myocardial protein kinase G activity in heart failure with preserved ejection fraction. *Circulation* 2012;126:830-9. DOI PubMed
42. Kumar AA, Kelly DP, Chirinos JA. Mitochondrial dysfunction in heart failure with preserved ejection fraction. *Circulation* 2019;139:1435-50. DOI PubMed PMC
43. Forman DE, Cittadini A, Azhar G, Douglas PS, Wei JY. Cardiac morphology and function in senescent rats: gender-related differences. *J Am Coll Cardiol* 1997;30:1872-7. DOI PubMed
44. Hung CL, Gonçalves A, Shah AM, Cheng S, Kitzman D, Solomon SD. Age- and sex-related influences on left ventricular mechanics in elderly individuals free of prevalent heart failure: The ARIC study (atherosclerosis risk in communities). *Circ Cardiovasc Imaging* 2017;10:e004510. DOI PubMed PMC
45. Liu CY, Liu YC, Wu C, et al. Evaluation of age-related interstitial myocardial fibrosis with cardiac magnetic resonance contrast-enhanced T1 mapping: MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol* 2013;62:1280-7. DOI PubMed PMC
46. Grilo GA, Shaver PR, Stoffel HJ, et al. Age- and sex-dependent differences in extracellular matrix metabolism associate with cardiac functional and structural changes. *J Mol Cell Cardiol* 2020;139:62-74. DOI PubMed
47. Petrov G, Regitz-Zagrosek V, Lehmkuhl E, et al. Regression of myocardial hypertrophy after aortic valve replacement: faster in women? *Circulation* 2010;122:S23-8. DOI PubMed
48. Achkar A, Saliba Y, Fares N. Differential gender-dependent patterns of cardiac fibrosis and fibroblast phenotypes in aging mice. *Oxid Med Cell Longev* 2020;2020:8282157. DOI PubMed PMC
49. Yusifov A, Chhatre VE, Koplin EK, et al. Transcriptomic analysis of cardiac gene expression across the life course in male and female mice. *Physiol Rep* 2021;9:e14940. DOI PubMed PMC
50. Banerjee SK, Wiener J. Effects of aging on atrial and ventricular human myosin. *Basic Res Cardiol* 1983;78:685-94. DOI PubMed
51. Biesiadecki BJ, Tachampa K, Yuan C, Jin JP, de Tombe PP, Solaro RJ. Removal of the cardiac troponin I N-terminal extension improves cardiac function in aged mice. *J Biol Chem* 2010;285:19688-98. DOI PubMed PMC
52. Effron MB, Bhatnagar GM, Spurgeon HA, Ruaño-Arroyo G, Lakatta EG. Changes in myosin isoenzymes, ATPase activity, and contraction duration in rat cardiac muscle with aging can be modulated by thyroxine. *Circ Res* 1987;60:238-45. DOI PubMed
53. Howlett SE. Age-associated changes in excitation-contraction coupling are more prominent in ventricular myocytes from male rats than in myocytes from female rats. *Am J Physiol Heart Circ Physiol* 2010;298:H659-70. DOI PubMed
54. Kane AE, Bisset ES, Keller KM, Ghimire A, Pyle WG, Howlett SE. Age, sex and overall health, measured as frailty, modify myofilament proteins in hearts from naturally aging mice. *Sci Rep* 2020;10:10052. DOI PubMed PMC
55. Pan B, Xu ZW, Xu Y, et al. Diastolic dysfunction and cardiac troponin I decrease in aging hearts. *Arch Biochem Biophys* 2016;603:20-8. DOI PubMed PMC
56. Pandya K, Kim HS, Smithies O. Fibrosis, not cell size, delineates beta-myosin heavy chain reexpression during cardiac hypertrophy and normal aging in vivo. *Proc Natl Acad Sci U S A* 2006;103:16864-9. DOI PubMed PMC
57. Ravichandran VS, Patel HJ, Pagani FD, Westfall MV. Cardiac contractile dysfunction and protein kinase C-mediated myofilament phosphorylation in disease and aging. *J Gen Physiol* 2019;151:1070-80. DOI PubMed PMC
58. Wannenburg T, Khan AS, Sane DC, Willingham MC, Faucette T, Sonntag WE. Growth hormone reverses age-related cardiac myofilament dysfunction in rats. *Am J Physiol Heart Circ Physiol* 2001;281:H915-22. DOI PubMed
59. Feridooni HA, Kane AE, Ayaz O, et al. The impact of age and frailty on ventricular structure and function in C57BL/6J mice. *J*

Physiol 2017;595:3721-42. DOI PubMed PMC
60. Nance ME, Whitfield JT, Zhu Y, et al. Attenuated sarcomere lengthening of the aged murine left ventricle observed using two-photon fluorescence microscopy. *Am J Physiol Heart Circ Physiol* 2015;309:H918-25. DOI PubMed PMC
61. Salcan S, Bongardt S, Monteiro Barbosa D, et al. Elastic titin properties and protein quality control in the aging heart. *Biochim Biophys Acta Mol Cell Res* 2020;1867:118532. DOI PubMed
62. Ayaz O, Banga S, Heinze-Milne S, Rose RA, Pyle WG, Howlett SE. Long-term testosterone deficiency modifies myofilament and calcium-handling proteins and promotes diastolic dysfunction in the aging mouse heart. *Am J Physiol Heart Circ Physiol* 2019;316:H768-80. DOI PubMed
63. Bupha-Intr T, Oo YW, Wattanapermpool J. Increased myocardial stiffness with maintenance of length-dependent calcium activation by female sex hormones in diabetic rats. *Am J Physiol Heart Circ Physiol* 2011;300:H1661-8. DOI PubMed
64. Bupha-Intr T, Wattanapermpool J. Regulatory role of ovarian sex hormones in calcium uptake activity of cardiac sarcoplasmic reticulum. *Am J Physiol Heart Circ Physiol* 2006;291:H1101-8. DOI PubMed
65. Fares E, Pyle WG, Ray G, et al. The impact of ovariectomy on calcium homeostasis and myofilament calcium sensitivity in the aging mouse heart. *PLoS One* 2013;8:e74719. DOI PubMed PMC
66. Fernandes RD, Hall A, Ferguson M, Lorenzen-Schmidt I, Balasubramaniam V, Pyle WG. Cardiac changes during the peri-menopausal period in a VCD-induced murine model of ovarian failure. *Acta Physiol (Oxf)* 2019;227:e13290. DOI PubMed PMC
67. Wadthaisong M, Witayavanitkul N, Bupha-Intr T, Wattanapermpool J, de Tombe PP. Chronic high-dose testosterone treatment: impact on rat cardiac contractile biology. *Physiol Rep* 2019;7:e14192. DOI PubMed PMC
68. Wattanapermpool J. Increase in calcium responsiveness of cardiac myofilament activation in ovariectomized rats. *Life Sciences* 1998;63:955-64. DOI PubMed
69. Wadthaisong M, Wattanapermpool J, de Tombe PP, Bupha-Intr T. Suppression of myofilament cross-bridge kinetic in the heart of orchidectomized rats. *Life Sci* 2020;261:118342. DOI PubMed
70. Arnold AP. Promoting the understanding of sex differences to enhance equity and excellence in biomedical science. *Biol Sex Differ* 2010;1:1. DOI PubMed PMC
71. Flurkey K, Randall PK, Sinha YN, Ermini M, Finch CE. Transient shortening of estrous cycles in aging C57BL/6J mice: effects of spontaneous pseudopregnancy, progesterone, L-dihydroxyphenylalanine, and hydergine. *Biol Reprod* 1987;36:949-59. DOI PubMed
72. Bustamante M, Garate-Carrillo A, R Ito B, et al. Unmasking of oestrogen-dependent changes in left ventricular structure and function in aged female rats: a potential model for pre-heart failure with preserved ejection fraction. *J Physiol* 2019;597:1805-17. DOI PubMed PMC
73. Zhao D, Guallar E, Ouyang P, et al. Endogenous sex hormones and incident cardiovascular disease in post-menopausal women. *J Am Coll Cardiol* 2018;71:2555-66. DOI PubMed PMC
74. Iorga A, Cunningham CM, Moazeni S, Ruffenach G, Umar S, Eghbali M. The protective role of estrogen and estrogen receptors in cardiovascular disease and the controversial use of estrogen therapy. *Biol Sex Differ* 2017;8:33. DOI PubMed PMC
75. Grohé C, Kahlert S, Löbbert K, et al. Cardiac myocytes and fibroblasts contain functional estrogen receptors. *FEBS Letters* 1997;416:107-12. DOI PubMed
76. Bhuiyan MS, Shioda N, Fukunaga K. Ovariectomy augments pressure overload-induced hypertrophy associated with changes in Akt and nitric oxide synthase signaling pathways in female rats. *Am J Physiol Endocrinol Metab* 2007;293:E1606-14. DOI PubMed
77. Rossouw JE, Anderson GL, Prentice RL, et al; Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA* 2002;288:321-33. DOI PubMed
78. Jazbutyte V, Hu K, Kruchten P, et al. Aging reduces the efficacy of estrogen substitution to attenuate cardiac hypertrophy in female spontaneously hypertensive rats. *Hypertension* 2006;48:579-86. DOI PubMed
79. Gurrala R, Kilanowski-Doroh IM, Hutson DD, et al. Alterations in the estrogen receptor profile of cardiovascular tissues during aging. *Geroscience* 2021;43:433-42. DOI PubMed PMC
80. Feldman HA, Longcope C, Derby CA, et al. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. *J Clin Endocrinol Metab* 2002;87:589-98. DOI PubMed
81. Zumoff B, Strain GW, Miller LK, Rosner W. Twenty-four-hour mean plasma testosterone concentration declines with age in normal premenopausal women. *J Clin Endocrinol Metab* 1995;80:1429-30. DOI PubMed
82. Fujita S, Chiba M, Ohta M, et al (1990) Alteration of plasma sex hormone levels associated with old age and its effect on hepatic drug metabolism in rats. J Pharmacol Exp Ther. PubMed
83. Dobrzycki S, Korecki J, Serwatka W, et al (2004) Assessment of possible correlations between endogenous androgens and the extent of coronary heart disease and left ventricle function. Przegla,d Lek 61:876–9. Available from: https://pubmed.ncbi.nlm.nih.gov/15789922/ [Last accessed on 15 Mar 2022].
84. Zhao S, Li X. The association of low plasma testosterone level with coronary artery disease in Chinese men. *Int J Cardiol* 1998;63:161-4. DOI PubMed
85. Marsh JD, Lehmann MH, Ritchie RH, Gwathmey JK, Green GE, Schiebinger RJ. Androgen receptors mediate hypertrophy in cardiac myocytes. *Circulation* 1998;98:256-61. DOI PubMed
86. Sebag IA, Gillis MA, Calderone A, et al. Sex hormone control of left ventricular structure/function: mechanistic insights using echocardiography, expression, and DNA methylation analyses in adult mice. *Am J Physiol Heart Circ Physiol* 2011;301:H1706-15. DOI PubMed
87. Rasmussen JJ, Schou M, Madsen PL, et al. Cardiac systolic dysfunction in past illicit users of anabolic androgenic steroids. *Am Heart*

J 2018;203:49-56. DOI PubMed

88. Bhasin S, Lincoff AM, Basaria S, et al; TRAVERSE Study Investigators. Effects of long-term testosterone treatment on cardiovascular outcomes in men with hypogonadism: Rationale and design of the TRAVERSE study. *Am Heart J* 2022;245:41-50. DOI PubMed
89. Ayaz O, Howlett SE. Testosterone modulates cardiac contraction and calcium homeostasis: cellular and molecular mechanisms. *Biol Sex Differ* 2015;6:9. DOI PubMed PMC
90. Bell JR, Mellor KM, Wollermann AC, et al. Aromatase deficiency confers paradoxical postischemic cardioprotection. *Endocrinology* 2011;152:4937-47. DOI PubMed
91. Jazbutyte V, Stumpner J, Redel A, et al. Aromatase inhibition attenuates desflurane-induced preconditioning against acute myocardial infarction in male mouse heart in vivo. *PLoS One* 2012;7:e42032. DOI PubMed PMC
92. Laakkonen EK, Karppinen JE, Lehti S, et al. Associations of sex hormones and hormonal status with arterial stiffness in a female sample from reproductive years to menopause. *Front Endocrinol (Lausanne)* 2021;12:765916. DOI PubMed PMC
93. Maluleke TT, Millen AME, Michel FS. The effects of estrogen deficiency and aging on myocardial deformation and motion in normotensive female rats. *Menopause* 2021;29:89-95. DOI PubMed
94. Shi W, Sheng X, Dorr KM, et al. Cardiac proteomics reveals sex chromosome-dependent differences between males and females that arise prior to gonad formation. *Dev Cell* 2021;56:3019-3034.e7. DOI PubMed
95. Charchar FJ, Bloomer LD, Barnes TA, et al. Inheritance of coronary artery disease in men: an analysis of the role of the Y chromosome. *Lancet* 2012;379:915-22. DOI PubMed PMC
96. Van PL, Bakalov VK, Bondy CA. Monosomy for the X-chromosome is associated with an atherogenic lipid profile. *J Clin Endocrinol Metab* 2006;91:2867-70. DOI PubMed
97. Arnold AP, Cassis LA, Eghbali M, Reue K, Sandberg K. Sex hormones and sex chromosomes cause sex differences in the development of cardiovascular diseases. *Arterioscler Thromb Vasc Biol* 2017;37:746-56. DOI PubMed PMC
98. Link JC, Chen X, Prien C, et al. Increased high-density lipoprotein cholesterol levels in mice with XX versus XY sex chromosomes. *Arterioscler Thromb Vasc Biol* 2015;35:1778-86. DOI PubMed PMC
99. Ji H, Zheng W, Wu X, et al. Sex chromosome effects unmasked in angiotensin II-induced hypertension. *Hypertension* 2010;55:1275-82. DOI PubMed PMC
100. Li J, Chen X, McClusky R, et al. The number of X chromosomes influences protection from cardiac ischaemia/reperfusion injury in mice: one X is better than two. *Cardiovasc Res* 2014;102:375-84. DOI PubMed PMC
101. Möller-Leimkühler AM (2007). Gender differences in cardiovascular disease and comorbid depression. *Dialogues Clin Neurosci* 2007;9:71-83. PubMed PMC
102. Mosca L, Banka CL, Benjamin EJ, et al; Expert Panel/Writing Group; American Academy of Physician Assistants; American Association for Clinical Chemistry; American Association of Cardiovascular and Pulmonary Rehabilitation; American College of Chest Physicians; American College of Emergency Physicians; American Diabetes Association; American Geriatrics Society; American Society for Preventive Cardiology; American Society of Echocardiography; American Society of Nuclear Cardiology; Association of Women's Health; Obstetric and Neonatal Nurses; Global Alliance for Women's Health. , Mended Hearts, Inc, National Black Nurses Association, National Black Women's Health Imperative, National Women's Health Resource Center, North American Menopause Society, Partnership for Gender-Specific Medicine at Columbia University, Preventive Cardiovascular Nurses Association, Society for Vascular Medicine and Biology, Society for Women's Health Research, Society of Geriatric Cardiology, Women in Thoracic Surgery, WomenHeart: the National Coalition for Women with Heart Disease. Evidence-based guidelines for cardiovascular disease prevention in women: 2007 update. *J Am Coll Cardiol* 2007;49:1230-50. DOI PubMed
103. Mann DL, Bristow MR. Mechanisms and models in heart failure: the biomechanical model and beyond. *Circulation* 2005;111:2837-49. DOI PubMed
104. Brodde OE, Bruck H, Leineweber K. Cardiac adrenoceptors: physiological and pathophysiological relevance. *J Pharmacol Sci* 2006;100:323-37. DOI PubMed
105. Fowler MB, Laser JA, Hopkins GL, Minobe W, Bristow MR. Assessment of the beta-adrenergic receptor pathway in the intact failing human heart: progressive receptor down-regulation and subsensitivity to agonist response. *Circulation* 1986;74:1290-302. DOI PubMed
106. Frielle T, Collins S, Daniel KW, Caron MG, Lefkowitz RJ, Kobilka BK. Cloning of the cDNA for the human beta 1-adrenergic receptor. *Proc Natl Acad Sci U S A* 1987;84:7920-4. DOI PubMed PMC
107. Kobilka BK, Dixon RA, Frielle T, et al. cDNA for the human beta 2-adrenergic receptor: a protein with multiple membrane-spanning domains and encoded by a gene whose chromosomal location is shared with that of the receptor for platelet-derived growth factor. *Proc Natl Acad Sci U S A* 1987;84:46-50. DOI PubMed PMC
108. Gauthier C, Tavernier G, Charpentier F, Langin D, Le Marec H. Functional beta3-adrenoceptor in the human heart. *J Clin Invest* 1996;98:556-62. DOI PubMed PMC
109. Rockman HA, Koch WJ, Lefkowitz RJ. Seven-transmembrane-spanning receptors and heart function. *Nature* 2002;415:206-12. DOI PubMed
110. Sato PY, Chuprun JK, Schwartz M, Koch WJ. The evolving impact of g protein-coupled receptor kinases in cardiac health and disease. *Physiol Rev* 2015;95:377-404. DOI PubMed PMC
111. Ferrara N, Komici K, Corbi G, et al. β-adrenergic receptor responsiveness in aging heart and clinical implications. *Front Physiol* 2014;4:396. DOI PubMed PMC
112. White M, Roden R, Minobe W, et al. Age-related changes in beta-adrenergic neuroeffector systems in the human heart. *Circulation*

1994;90:1225-38. DOI PubMed

113. Xiao RP, Tomhave ED, Wang DJ, et al. Age-associated reductions in cardiac beta1- and beta2-adrenergic responses without changes in inhibitory G proteins or receptor kinases. *J Clin Invest* 1998;101:1273-82. DOI PubMed PMC
114. Freedman NJ, Liggett SB, Drachman DE, Pei G, Caron MG, Lefkowitz RJ. Phosphorylation and desensitization of the human beta 1-adrenergic receptor. Involvement of G protein-coupled receptor kinases and cAMP-dependent protein kinase. *J Biol Chem* 1995;270:17953-61. DOI PubMed
115. Ferrara N, Ogara P, Wynne D, et al. Decreased contractile responses to isoproterenol in isolated cardiac myocytes from ageing guinea-pigs. *J Mol Cell Cardiol* 1995;27:1141-50. DOI PubMed
116. Silver MA, Horton DP, Ghali JK, Elkayam U. Effect of nesiritide versus dobutamine on short-term outcomes in the treatment of patients with acutely decompensated heart failure. *J Am Coll Cardiol* 2002;39:798-803. DOI PubMed
117. Baxter AJ, Spensley A, Hildreth A, Karimova G, O'Connell JE, Gray CS. Beta blockers in older persons with heart failure: tolerability and impact on quality of life. *Heart* 2002;88:611-4. DOI PubMed PMC
118. Wheatley CM, Snyder EM, Johnson BD, Olson TP. Sex differences in cardiovascular function during submaximal exercise in humans. *Springerplus* 2014;3:445. DOI PubMed PMC
119. Yusifov A, Chhatre VE, Zumo JM, et al. Cardiac response to adrenergic stress differs by sex and across the lifespan. *Geroscience* 2021;43:1799-813. DOI PubMed PMC
120. Young ME, McNulty P, Taegtmeyer H. Adaptation and maladaptation of the heart in diabetes: Part II: potential mechanisms. *Circulation* 2002;105:1861-70. DOI PubMed
121. Monasky MM, Varian KD, Janssen PM. Gender comparison of contractile performance and beta-adrenergic response in isolated rat cardiac trabeculae. *J Comp Physiol B* 2008;178:307-13. DOI PubMed
122. Vizgirda VM, Wahler GM, Sondgeroth KL, Ziolo MT, Schwertz DW. Mechanisms of sex differences in rat cardiac myocyte response to beta-adrenergic stimulation. *Am J Physiol Heart Circ Physiol* 2002;282:H256-63. DOI PubMed
123. Schwertz DW, Vizgirda V, Solaro RJ, Piano MR, Ryjewski C. Sexual dimorphism in rat left atrial function and response to adrenergic stimulation. *Mol Cell Biochem* 1999;200:143-53. DOI PubMed
124. Lujan HL, Dicarlo SE. Sex differences to myocardial ischemia and beta-adrenergic receptor blockade in conscious rats. *Am J Physiol Heart Circ Physiol* 2008;294:H1523-9. DOI PubMed
125. Lujan HL, Kramer VJ, DiCarlo SE. Sex influences the susceptibility to reperfusion-induced sustained ventricular tachycardia and beta-adrenergic receptor blockade in conscious rats. *Am J Physiol Heart Circ Physiol* 2007;293:H2799-808. DOI PubMed
126. McIntosh VJ, Chandrasekera PC, Lasley RD. Sex differences and the effects of ovariectomy on the β-adrenergic contractile response. *Am J Physiol Heart Circ Physiol* 2011;301:H1127-34. DOI PubMed PMC
127. Curl CL, Wendt IR, Kotsanas G. Effects of gender on intracellular. *Pflugers Arch* 2001;441:709-16. DOI PubMed
128. Sun J, Picht E, Ginsburg KS, Bers DM, Steenbergen C, Murphy E. Hypercontractile female hearts exhibit increased S-nitrosylation of the L-type Ca2+ channel alpha1 subunit and reduced ischemia/reperfusion injury. *Circ Res* 2006;98:403-11. DOI PubMed
129. Chu SH, Sutherland K, Beck J, Kowalski J, Goldspink P, Schwertz D. Sex differences in expression of calcium-handling proteins and beta-adrenergic receptors in rat heart ventricle. *Life Sci* 2005;76:2735-49. DOI PubMed
130. Parks RJ, Ray G, Bienvenu LA, Rose RA, Howlett SE. Sex differences in SR Ca(2+) release in murine ventricular myocytes are regulated by the cAMP/PKA pathway. *J Mol Cell Cardiol* 2014;75:162-73. DOI PubMed
131. Bordallo J, Cantabrana B, Suárez L, Sánchez M. Testosterone inhibits cAMP-phosphodiesterases in heart extracts from rats and increases cAMP levels in isolated left atria. *Pharmacology* 2011;87:155-60. DOI PubMed