

# Sex Differences in Cardiovascular Aging and Heart Failure

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## Abstract

**Purpose of the Review** This review summarizes sex-related changes in the heart and vasculature that occur with aging, both in the presence and absence of cardiovascular disease (CVD).

**Recent Findings** In the presence of CVD risk factors and/or overt CVD, sex-specific changes in the number of cardiomyocytes, extent of the myocardial extracellular matrix, and myocellular hypertrophy promote unique patterns of LV remodeling in men and women. In addition, age- and sex-specific vascular stiffening is also well established, driven by changes in endothelial dysfunction, elastin–collagen content, microvascular dysfunction, and neurohormonal signaling. Together, these changes in LV chamber geometry and morphology, coupled with heightened vascular stiffness, appear to drive both age-related increases in systolic function and declines in diastolic function, particularly in postmenopausal women. Accordingly, estrogen has been implicated as a key mediator, given its direct vasodilating properties, association with nitric oxide excretion, and involvement in myocellular Ca<sup>2+</sup> handling, mitochondrial energy production, and oxidative stress.

**Summary** The culmination of the abovementioned sex-specific cardiac and vascular changes across the lifespan provides important insight into heart failure development, particularly of the preserved ejection fraction variety, while offering promise for future preventive strategies and therapeutic approaches.

**Keywords** Sex · Aging · Heart failure · Cardiovascular disease

## Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide, accounting for 31% of all reported deaths in 2016 [1]. Moreover, nearly 81% of all CVD-attributable deaths were among individuals  $\geq 65$  years old [2], reinforcing the

notion that CVD is predominantly a disease of senescence. While this general pattern is true for both men and women, important sex-specific differences exist. For example, according to the National Health and Nutrition Examination Survey, collected from 2013 to 2016, the prevalence of CVD was lower in premenopausal women compared with age-matched men, yet surpassed that of men after menopause [2]. Despite near universal recognition of this sex-by-age interaction, however, the exact mechanism(s) by which age and sex influence CVD development and progression remains elusive. This review summarizes sex-related changes in the heart and vasculature that occur with aging, both in the presence and absence of cardiovascular disease.

## Age–Sex Interaction and the Heart

### Left Ventricular Structural Remodeling

Both sex and age are known to impact cardiac morphology (Fig. 1). Data from both the Framingham Heart Study and Multi-Ethnic study of Atherosclerosis (MESA) demonstrate

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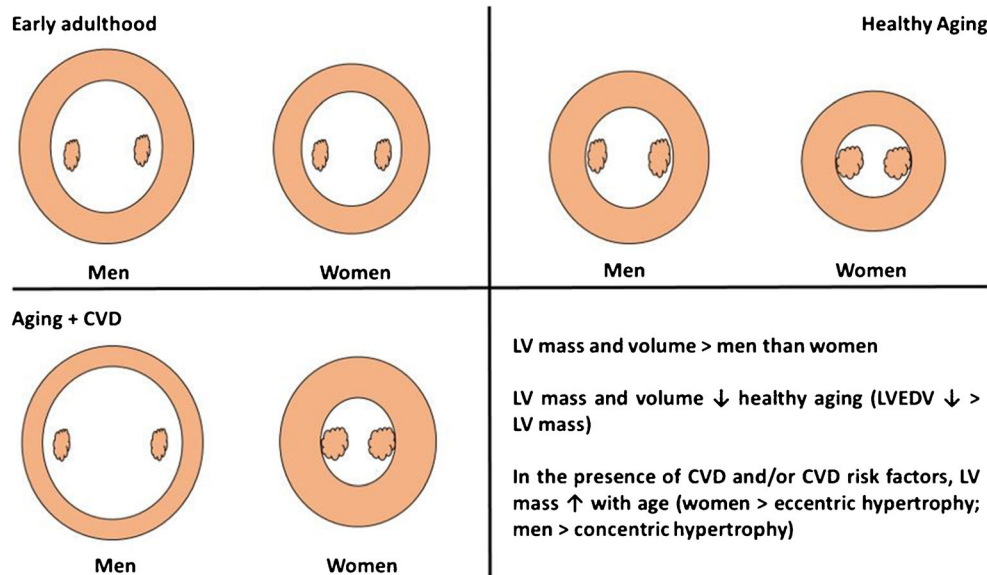
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**Fig. 1** Influence of age, sex, and cardiovascular disease on left ventricular structure. Conceptual left ventricular cross-sectional image, at the level of the papillary muscles, showing the most common structural adaptations of the left ventricle (LV) in men and women across the lifespan and in the presence of cardiovascular disease (CVD) or CVD risk factors. Beginning with early adulthood, LV mass and volume are greater in

men than women. With healthy aging, mass and volume decline, with LVEDV declining to a greater extent than LV mass, resulting in more concentric hypertrophy. In the presence of CVD risk factors and/or CVD, women tend to develop an eccentric pattern of hypertrophy, while men tend to develop a concentric pattern of hypertrophy

that left ventricular (LV) mass and volume are significantly greater in men than women, even after adjusting for height and body surface area (BSA) [3, 4]. Across the lifespan, absolute LV mass and LVEDV tend to decrease with healthy aging, with LVEDV declining more steeply with age, resulting in a progressive rise in LV concentricity over time [4–8].

In contrast to healthy aging, however, in the presence of CVD risk factors and/or overt CVD, LV mass increases with age and is associated with sex-specific cardiac remodeling, such that women experience greater concentric hypertrophy, while men tend to develop an eccentric pattern of hypertrophy [9, 10]. Indeed, studies of chronic pressure overload by aortic stenosis have found that women demonstrate more concentric remodeling and less eccentric hypertrophy than men [11–16]. Likewise, in a large dataset of 3,745 women and men undergoing both cMRI and invasive coronary angiography, women presented with greater concentric remodeling and less eccentric hypertrophy [17•]. Extrapolating these sex-specific patterns of remodeling may provide insight into disease risk and pathology, where women are two times more likely to develop heart failure with preserved ejection fraction (HFpEF) than men, a condition associated with a clustering of CVD risk factors, and adverse left ventricular remodeling [18, 19]. While LV concentric remodeling is only present in a fraction of the HFpEF population [20], it is overrepresented in women compared with men with HFpEF [21].

At the cellular level, these patterns may be explained by cardiomyocyte loss, an increase in extracellular matrix, and myocellular hypertrophy [22–25]. Indeed, aging is associated

with progressive neurohumoral dysfunction that contributes to cardiomyocyte death [26–31] in a sex-specific manner [32]. Among autopsies of 53 men and 53 women, cardiomyocyte death with healthy aging occurs to a greater extent in men than women [33]. This sex difference likely arises from (A) a larger pool of cardiac stem cells in women that allow for greater myocyte turnover compared with men [34] and (B) sex-specific rates of apoptosis. Regarding the latter, men experience greater rates of apoptosis than women when free of any cardiovascular disease [35], after acute myocardial infarction [36], and in heart failure [37].

Collagen content in the human heart nearly doubles over the lifespan (from ~3.9 to ~5.9%), independent of pathology [38]. This results in progressive reductions in LV compliance [39, 40] and is regarded as a primary mechanism of age-related diastolic dysfunction [22], discussed in more detail in the following section, “**Left Ventricular Diastolic Function.**” Whether an age-by-sex interaction exists with collagen content in the heart, however, remains unclear. LV systolic and diastolic stiffness is indeed greater in women than men across the lifespan, with an apparent acceleration of LV systolic and diastolic elastance in women beyond 50 years of age [41–43]. In the presence of CVD risk factors, the MESA demonstrated that LV extracellular volume (ECV), measured by gadolinium-enhanced MRI, is greater in women than men until ~84 years of age [44]. Likewise, ECV is elevated to a greater extent in women than men with mild aortic stenosis, despite women having fewer comorbidities [45]. To what extent these imaging-based observations reflect expansion of

extracellular proteins, however, remains incompletely understood, as biopsy studies have reported opposite results among patients with aortic stenosis [11, 12]. In line with this later observation, a recent imaging study found greater ECV in healthy young women compared with age- and health-matched men, together with greater myocardial blood volume and myocardial resting and peak perfusion, suggesting that women may have greater capillary density, rather than a more developed extracellular matrix per se [46•]. More work is therefore needed to better define the age-by-sex interaction of extracellular proteins like collagen and specific mechanisms driving morphological changes over the lifespan and in the presence of CVD.

### Left Ventricular Systolic Function

Clinically, LV ejection fraction (LVEF) is the most widely used measure to assess systolic function, despite its well-recognized shortcomings. Consistent with the age- and sex-related LV structural changes described previously, LVEF tends to be higher in women than men [47, 48], with studies reporting both increases [49–51] and decreases [52–57] in LVEF with advancing age; the latter of which affecting men more than women. As summarized in Table 1, similar observations have also been made with more advanced measures of LV contractility [41, 60, 61], regional tissue deformation indices [62, 63], and twist mechanics [64–66].

Sex hormones seem to be an unlikely source of this age-related rise in systolic function, given that both estrogen and testosterone decline with age. This is not to suggest that estrogen and testosterone are not involved in the mechanical and protein function of ventricular myocytes, which they undoubtedly are [67–77], just that their role in the age-associated rise in systolic function seems improbable. Indeed, ovarian hormone deficiency decreases (not increases) myocellular contractile function, and while this function is often restored with estrogen replacement [68–77], this fails to explain the rise in systolic function often observed in postmenopausal women. Likewise, while testosterone is strongly implicated in the density of L-type  $\text{Ca}^{2+}$  channels, sarcoplasmic reticulum  $\text{Ca}^{2+}$  availability, the magnitude of the  $\text{Ca}^{2+}$  transient, and the maximal myofilament responsiveness [67], it seems unlikely to explain the heightened systolic function observed with age, especially considering that testosterone levels decline with age, in both men and women [78–80]. In fact, given that the age-associated rise in systolic function is attenuated in men compared with women, for whom testosterone plays a much more dominant role across the lifespan, argues against testosterone being a contributory mechanism.

In contrast to the sex hormone hypothesis, many believe that this “heightened” contractility is reflective of a necessary adaptation to maintain optimal output in the face of higher resistance. Indeed, it is now well established that large-artery

stiffness increases with age [41, 81–83] and is higher in women [41, 81, 84–86], independent of vascular disease or risk factors [41, 81, 82, 87]. To maintain optimal output, the left ventricle must therefore develop greater systolic stiffness [88–92]. That end-systolic elastance is elevated in women, particularly in older women, supports this interpretation [41, 59]. The exact mechanism for this augmented systolic performance, however, remains incompletely understood. To date, there is no clear evidence for heightened inotropy (e.g. circulating catecholamines, calcium affinity, etc.). Instead, alterations in chamber geometry with age and sex is likely to play the most dominant role. In accordance with the left ventricle’s unique helical muscle fiber orientation, contraction of the endocardial fibers contributes to longitudinal shortening, while contraction of the epicardial fibers contributes to circumferential shortening and left ventricular twist [93]. Age, along with presence of cardiovascular risk factors with/without overt structural remodeling, is associated with impaired subendocardial function [94], giving rise to reduced global longitudinal shortening [95, 96], for which arterial stiffness is a likely contributor [97], particularly in women [98]. At the same time, subepicardial function remains relatively unaffected, allowing for the longer lever arm of the epicardial fibers to dominate, resulting in increased circumferential shortening and increased left ventricular twist, together of which help to maintain (and even augment) left ventricular ejection fraction [99, 100] (Fig. 2). Accordingly, given the structural changes that occur with age (i.e. concentric remodeling, subendocardial dysfunction, sphericity), especially in women, and in the presence of cardiovascular disease/risk factors [4, 41, 64, 94, 101, 102], mechanical factors seem to play the most influential role.

### Left Ventricular Diastolic Function

Both age and female sex are associated with increased LV stiffness, related to concentric remodeling, increased collagen deposition, and loss of estrogen. As a result, the LV end-diastolic pressure–volume relationship is shifted leftward with healthy aging [39, 40], a result which is augmented in elderly females [103]. Moreover, age and female sex appear to affect other components of diastole, including early and late diastolic filling patterns [58, 59, 104, 105], as summarized in Table 2.

The majority of results to date suggest that postmenopausal status is strongly related to impaired LV relaxation, with most population-based studies showing accelerated age-related impairments in LV relaxation in women after 50 years of age (the average onset of natural menopause). While the exact mechanism for this age-by-sex interaction remains incompletely understood, estrogen is likely a key mediator for age-related diastolic dysfunction in women. Indeed, estrogen is a direct vasodilator [106, 107]; it promotes nitric oxide excretion [108,

**Table 1** Summary of literature evaluating the influence of age and sex on left ventricular systolic function

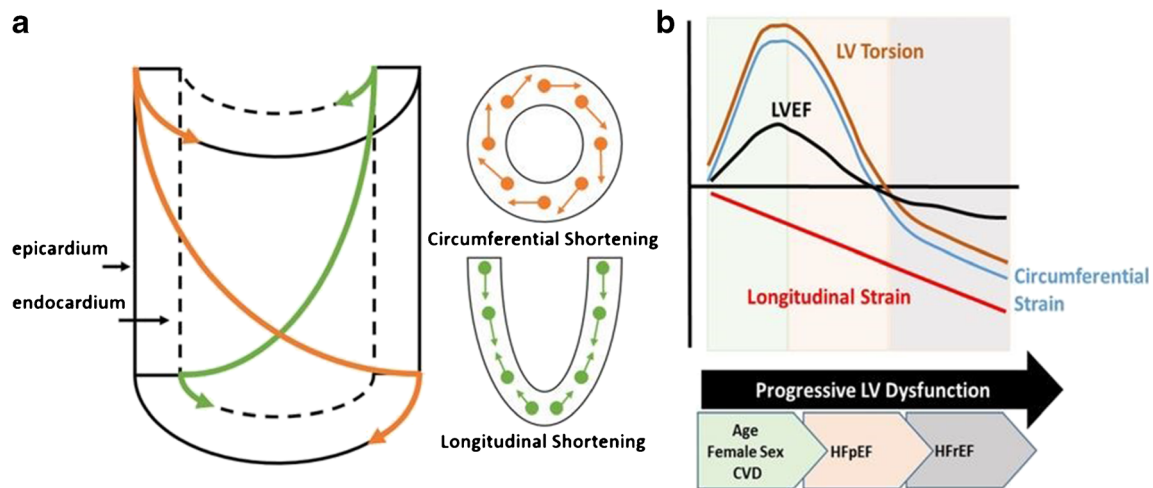
First author, year	Subjects	Age (years)	Results
Redfield et al., 2005 [41]	2042 subjects (984 men, 1058 women)	≥ 45	Age: ↑ end-systolic elastance (Ees) Age × Sex: adjusted for age, LV systolic elastance was higher in women than men. LV systolic elastance increased with age in men and women, but more steeply in women. Ees adjusted for chamber size (LVEDV) increased with age, but not when sex was added to the model.
Hayashi et al., 2015 [58]	265 subjects without abnormal clinical, electrocardiographic, and echocardiographic findings	20–89	Age: s' ↔ Sex: s' ↔ Age × Sex: none
Hoshida et al., 2016 [59]	479 hypertensive subjects (267 men, 212 women)	< 65, 65–74, > 75	Age: ↔ EF Sex: ↔ stress corrected fractional shortening (FS/Ees) Age × Sex: stress corrected fractional shortening (FS/Ees) related to age in women but not men
Hayward et al., 2001 [60]	30 subjects (14 men, 16 women) with normal LV function and no history of MI or HF	48–75	Age: not assessed Sex: women ↑ ESPVR and PRSWR Age × Sex: not assessed
Celentano et al., 2003 [61]	517 normotensive and hypertensive subjects with no history of CV or endocrinal disease	20–70	Age: not assessed Sex: normotensive and hypertensive stress-corrected mid wall shortening was higher in women than men, independent of LV geometry, body size, age, and heart rate Age × Sex: not assessed
Gruner Svealv et al., 2006 [62]	82 healthy subjects	20–29, 50–59, and 60–69	Age: LV systolic amplitude, LV maximal systolic velocity ↓; time to maximal systolic velocity ↑ Sex: AVP-FS ↑ in women, LVEF tended ( $p = 0.06$ ) to be ↑ in women Age × Sex: not assessed
Foll et al., 2010 [63]	62 healthy subjects (32 men, 30 women)	20–40, > 40–60, > 60	Age: ↓ peak systolic long axis velocity and peak systolic apical rotation, ↑ time to peak systolic long axis velocity, and time to peak apical systolic rotation Age × Sex: systolic long-axis velocity decreased to a greater extent in women
Yoneyama et al., 2012 [64]	1478 subjects (MESA)	45–54, 55–64, 65–74, 75–84	Age: torsion and LVEF ↑ Sex: torsion ↑ women than men Age × Sex: LV torsion increased with advancing age, and women had greater LV torsion than men in all age groups
Hung et al., 2017 [65]	1105 asymptomatic subjects	67–70, 71–73, 73–76, 76–80, 80–89	Age: ↓ longitudinal strain; ↑ circumferential strain, twist, and torsion Sex: ↑ longitudinal and circumferential strain, torsion, and twist in women vs. men Age × Sex: torsion increased with age in women > men. Global longitudinal strain decreased with age in women > men
Nio et al., 2017 [66]	82 healthy subjects	19–32, 45–58	Age: LV ejection fraction, twist, torsion, twist velocity, apical rotation ↑ Sex: LV ejection fraction, circumferential strain, circumferential strain rate ↑ women vs. men Age × Sex: apical rotation, apical rotational velocity, circumferential strain, and circumferential strain rate ↑ in men than women with age

LV left ventricle, EF ejection fraction, AVP-FS atrioventricular plane-fractional shortening, ESPVR end-systolic pressure volume relationship, PRSWR preload recruitable stroke work relationship

109] and directly impacts myocellular calcium ( $\text{Ca}^{2+}$ ) handling; all of which could impact diastolic performance.

Myocardial relaxation is inherently dependent on the removal of  $\text{Ca}^{2+}$  from the cytosol, primarily through

sarco(endo)plasmic reticulum  $\text{Ca}^{2+}$ -ATPase 2a (SERCA2a) uptake into the sarcoplasmic reticulum and sodium/calcium exchanger (NCX) extrusion from the cell [110, 111]. Although the effect of sex hormones on NCX remains



**Fig. 2** Twist mechanics and altered strain contributions to left ventricular ejection fraction with age, sex, and cardiovascular disease. **a** Left ventricular myofiber architecture, changing from a left-handed helix in the subepicardium to a right-handed helix in the subendocardium. Contraction of these two opposing myofiber layers gives rise to circumferential and longitudinal shortening about the long axis of the cylinder. Note the longer lever arm of the subepicardial fibers compared with the subendocardial fibers. When both layers contract simultaneously, the epicardial fibers have a mechanical advantage,

dominating the overall direction and magnitude of rotation. This mechanical advantage is augmented in conditions with impaired subendocardial function and/or a greater subepicardial radius (i.e. concentric hypertrophy). **b** Conceptual model illustrating patterns of change in left ventricular tissue deformation, twist mechanics, and ejection fraction through the onset of early mechanical dysfunction (associated with age, sex, and cardiovascular comorbidities), heart failure with preserved ejection fraction (HFpEF), and heart failure with reduced ejection fraction (HFrEF)

inconclusive, SERCA2a's response to hormonal changes has been well documented, at least in preclinical models of human aging. For example, ovariectomy (OVX) in middle and old age normotensive rats reduces phosphorylated phospholamban (PLB), responsible for facilitating SERCA2a activity, resulting in decreased lusitropy and increased cardiac filling pressures, with the older OVX rats experiencing the worst diastolic dysfunction. That G protein-coupled estrogen receptor (GPER) activation significantly improves LV lusitropy in this model—resulting in greater SERCA2a expression and reduced interstitial collagen content—strongly supports estrogen as a principle determinant of age-by-sex-related diastolic dysfunction [112]. Similar results have also been reported across different strains of OVX rats and experimental models, including direct GPER knockout in transgenic mice [70, 71, 113]. Furthermore, estrogen treatment in a well-established translational nonhuman primate model of menopause preserved diastolic function, in part, by modulating calcium homeostasis [114]. Although not always consistent, the beneficial effect of estrogen on diastolic function has also been demonstrated by hormone replacement therapy trials in women, as reviewed by Maslov and colleagues [115]. Less work has been performed evaluating the influence of male gonads and associated sex hormones, on diastolic (dys)function. In male mice, following bilateral removal of the testes (GDX), evidence of diastolic dysfunction has indeed been observed in both isolated myocyte preparations and in vivo [116]. It remains unclear, however, whether this effect is directly related to testosterone itself or the reduction in estradiol via aromatization of testosterone. While direct cardiomyocyte treatment

with testosterone influences  $\text{Ca}^{2+}$ -related gene expression [117], more work in this area is needed.

Diastole is a highly energy-dependent process [118]. Under normal conditions, the majority of ATP is produced from oxidative phosphorylation in the mitochondria. Impairments in ATP generation, whether from impaired oxygen delivery or oxidative phosphorylation, could therefore have direct effects on diastolic function. We and others have described clear sex differences in the presentation of myocardial ischemia, which often develops in the presence of age and/or cardiovascular risk factors. For a more detailed review of sex-specific patterns of myocardial ischemia, the reader is directed to the following comprehensive reviews: [119, 120]. While we have observed some evidence to support a role for myocardial ischemia in the development of diastolic dysfunction in women with signs and symptoms of ischemia with no obstructive coronary artery disease [121–123], investigations are currently underway to evaluate both the direct and indirect effect of myocardial ischemia on diastolic function in this burgeoning clinical population.

Abnormalities in mitochondrial energy production can also contribute to impaired diastolic function via oxidative stress, as is increasingly recognized in the pathogenesis of heart failure [124, 125]. Upon ischemia/reperfusion, female Sprague Dawley rat hearts express lower rates of ROS production compared with age-matched male hearts via posttranslational modification of mitochondrial proteins [126], with estrogen being strongly implicated as the principle cardioprotective agent. Indeed, preclinical ischemia/reperfusion studies incorporating OVX with and without exogenous estradiol

**Table 2** Summary of literature evaluating the influence of age and sex on left ventricular diastolic function

First author, year	Subjects	Age (years)	Results
Okura et al., 2009 [104]	1333 healthy subjects w/o known heart disease or hypertension	10–89	Age: E, E/A, $e'$ ↓; A, A', E/E' ↑ Sex: in young men and women (10–29 years) $e' \leftrightarrow$ ; $e' \uparrow$ premenopausal women than men (30–49 years); Age × Sex: $e' \uparrow$ men than women (70–79 years; i.e. accelerated age effect in older women)
Daimon et al., 2011 [105]	700 healthy Japanese volunteers	20–79	Age: E, E/A ratio, $e' \downarrow$ Sex: women < 50 years, ↑ E, E/A ratio, $e'$ than men Age × Sex: > 50 years, E, E/A ratio, $e' \leftrightarrow$ men and women, with significant age × sex interaction
Hayashi et al., 2015 [58]	265 subjects without abnormal clinical, electrocardiographic, and echocardiographic findings	20–89	Age: $e' \downarrow$ Sex: $e' \leftrightarrow$ , excepted for 40–59 years age group, women > men Age × Sex: none
Hayward et al., 2001 [60]	30 subjects (14 men, 16 women) with normal LV function and no history of MI or HF	48–75	Age: not assessed Sex: Women ↓ passive diastolic compliance Age × Sex: not assessed
Gruner Svealv et al., 2006 [62]	82 healthy subjects	20–29, 50–59, and 60–69	Age: LV early diastolic filling amplitude, LV maximal early diastolic filling velocity ↓; LA contraction amplitude, LA maximal contraction velocity, LA contraction time, LA filling fraction ↑ Sex: LA contraction amplitude, LA filling fraction, LA maximal contraction velocity ↓ women vs. men Age × Sex: not assessed
Foll et al., 2010 [63]	62 healthy subjects (32 men, 30 women)	20–40, > 40–60, > 60	Age: ↑ peak diastolic radial and long-axis velocity, ↑ time to peak diastolic radial and long-axis velocity Age × Sex: diastolic long-axis velocity decreased to a greater extent in women, ↓ time to peak apical diastolic rotation in aging women
Hung et al., 2017 [65]	1105 asymptomatic subjects	67–70, 71–73, 73–76, 76–80, 80–89	Age: ↑ LA volume, mitral inflow deceleration time, and E/e'; ↓ E/A ratio, $e'$ Sex: $e' \downarrow$ women vs. men Age × Sex: $e'$ declines more with age in women vs. men
Nio et al., 2017 [66]	82 healthy subjects	19–32, 45–58	Age: longer isovolumic relaxation times, slower early diastolic velocities (E and $e'$ ), with faster A and a'. Delayed time to peak untwisting, with lower peak diastolic apical circumferential strain rates. Sex: time to peak untwisting rate, basal circumferential diastolic strain rate was faster in women than men Age × Sex: time to peak untwisting velocity and time to peak basal and apical rotational velocity were later in men than women
Hoshida et al., 2016 [59]	479 hypertensive subjects (267 men, 212 women)	< 65, 65–74, > 75	Age: ↑ E/e' and E/e' adjusted for stroke volume index Age × Sex: women ≥ 75 years, ↑ E/e' and E/e' adjusted for stroke volume index

E early mitral inflow velocity, A late mitral inflow velocity,  $e'$  early annular tissue velocity, E/e' a surrogate measure of left ventricular end-diastolic pressure, LA left atrium

treatment suggest that estrogen promotes electron transport chain activity and ATP production [127], upregulates mitochondrial antioxidants [128], and downregulates mitochondrial apoptotic pathways [129]. With regard to cardiac pathology, estradiol treatment in an OVX mouse model of hypertrophic cardiomyopathy prevents energy dysregulation, reduces ROS formation, and improves diastolic function [130]. ROS production also serves as a scavenger to nitric oxide, a key regulator of normal diastolic function [131–136]. Indeed,

cardiomyocytes possess both the “endothelial” and “neuronal” isoforms of nitric oxide synthase (NOS), with neuronal NOS most strongly implicated in cardiac relaxation, via effects on phospholamban phosphorylation [132–136]. Uncoupling of NOS often occurs during oxidative depletion of its co-factor tetrahydrobiopterin (BH<sub>4</sub>), leading to production of superoxide instead of NO. Estrogen is known to modulate BH<sub>4</sub>, and therefore may represent a key source of diastolic dysfunction in aged postmenopausal women. For

example, OVX rats demonstrate reduced cardiac  $BH_4$  concentration, and  $BH_4$  treatment after OVX improves lusitropy and reduces cardiac filling pressures, collagen content, and ROS production [137].

As mentioned, estrogen is also a direct vasodilator of the arterial system [106, 107]. While this may explain at least part of the female-dominant pattern of nonobstructive coronary artery disease we and others have observed in middle-aged women [138–140], it may also provide insight into the accelerated impairment in early diastolic function seen in older women. For example, ventricular-arterial coupling is an important contributor to cardiac mechanics and hemodynamic control. Alterations in the stiffness of the central vascular system elevate cardiac afterload and compromise cardiac efficiency [141–143], with the added potential of decreasing coronary perfusion [144, 145]. While this mechanism of diastolic dysfunction has been implicated in hypertension, diabetes, and heart failure [146–148], the age-by-sex interaction of this proposed mechanism has not been well described, warranting further investigation.

## Age–Sex Interaction and the Vasculature

The vascular system is commonly divided into two levels: the macrovasculature and microvasculature. The macrovasculature is composed of large elastic arteries that buffer intermittent increases in pulsatility following left ventricular ejection and muscular arteries that serve as conduit vessels to supply blood to the microvasculature (< 300  $\mu\text{m}$  in diameter), for subsequent tissue perfusion and oxygenation. The microvasculature is therefore composed of arterioles, capillaries, and venules. As mentioned, vascular stiffness increases with age [81–83], independent of vascular disease or risk factors [81, 82, 87], and is higher in women [81, 84–86]. Multiple mechanisms have been proposed to explain age- and sex-dependent vascular stiffening, including endothelial dysfunction, changes in vascular protein composition (i.e. elastin–collagen content), microvascular dysfunction, and neurohormonal signaling, each of which is discussed in more detail herein.

### Endothelial (Dys)function

The vascular endothelium, a single-cell layer lining the inner lumen of all blood vessels, plays a pivotal role in blood flow regulation by synthesizing and secreting vasoactive molecules, principally nitric oxide (vasodilator) and endothelin-1 (vasoconstrictor).

Endothelium-dependent vasodilation may be invoked by either chemical (acetylcholine) or mechanical (increase in blood flow and shear stress) stimuli, the latter of which is the principle of flow-mediated dilation (FMD), an index of

coronary endothelial health/function [149] along with overall endothelial function. Both acetylcholine-mediated vasodilation and flow-mediated dilation (FMD) decrease with age in men, but remain preserved in women typically until the onset of menopause, after which endothelial dependent vasodilation markedly declines [150, 151]. In accordance with the biological timeline of these results, the majority of work strongly implicates estrogen and testosterone as primary mediators of endothelial-dependent vasodilation. Indeed, both estrogen and testosterone increase NO production via receptor-mediated activation of endothelial NO synthase. Accordingly, endothelial-dependent vasodilation declines with age in both men and women [150–153] and is attenuated in premenopausal women treated with a gonadotropin-releasing hormone antagonist (GnRH-ant) [154] and young men treated with an aromatase inhibitor, which blocks endogenous production of estrogen [155], and restored by estradiol treatment [154, 156–159]. Less clear is the role of testosterone in the regulation of endothelial function, as results from several cross-sectional studies evaluating FMD in men with low serum testosterone remain equivocal [160–164]. Both testosterone and estrogen possess antioxidant and anti-inflammatory properties that are lost in hormone-deficient states, regarded as the principle mechanism linking sex hormones with endothelial-dependent vasodilation [165–168].

Less established, but increasingly recognized, is the role of endothelin-1 on endothelial function both with aging and between sexes. Endothelin-1 is a potent vasoconstrictor produced and released by endothelial cells that acts on two receptor subtypes,  $ET_A$  and  $ET_B$ , located on the vascular smooth muscle [169]. In addition,  $ET_B$  receptors are also located on the endothelium and mediate vasodilation [169, 170]. Emerging evidence suggests that endothelin-1 receptors may be sexually dimorphic [171•] [172], with endothelin-1 preferentially binding to  $ET_B$  receptors in women [173]. Moreover, endothelin-1-mediated vasoconstriction appears to be augmented with age [174, 175], with  $ET_B$ -mediated vasodilation potentially lost in postmenopausal women [176]. Whether targeting endothelin receptors can improve cardiovascular disease outcomes, quality of life, and overall survival remains largely unknown, but with the advent of endothelin receptor antagonists, has great potential of being addressed within the next decade.

### Elastin–Collagen Content

Large blood vessels like the aorta are inherently “elastic,” facilitating blood vessel distension with each heartbeat (i.e. stroke volume), dampening velocity and pressure fluctuations, and maintaining consistent unidirectional blood flow. Vascular elasticity is predominantly mediated by the balance between collagen—a stiff scaffolding protein—and elastin—an elastic protein designed to facilitate the repetitive distention

of the vessel. In male rodents, aging is associated with a progressive shift in the collagen–elastin ratio, whereby elastin is degraded with age and collagen expression is increased [177–179]. To our knowledge, however, this work has not been replicated in female rodents, rendering our understanding of sex-by-age-related differences in vascular protein composition incomplete. In nonhuman primates, aortic stiffness increases with age to a greater extent in male versus female monkeys, attributable to preserved collagen but decreased elastin among old male monkeys that was larger in magnitude than that observed in old female monkeys [180]. Notably, several cross-species differences in specific collagen isoform changes with aging appear to exist, at least between mice and monkeys, highlighting the need to extend these observations to humans (of both sexes). Unfortunately, aside from a limited number of autopsy studies completed more than three decades ago [181–185], our clinical understanding remains limited. Nevertheless, despite several challenges with interpreting these early data, including issues surrounding differences in both the location of dissection (abdominal aorta vs thoracic) and prior health status of the individuals included, it does appear that human aging is indeed associated with a similar shift in collagen–elastin ratio. While the exact mechanism driving age (and potentially sex) related changes in the balance between collagen and elastin remains incompletely understood, reactive oxygen species and inflammation—which could degrade elastin and increase the deposition of collagen—are thought to play a major role [168]. More work is needed, however, to truly address this question.

### Microvascular (Dys)function

We and others have shown that coronary microvascular dysfunction (CMD) is more prevalent in women than men [186–188], and several reports of “microvascular dysfunction” in HFpEF have also recently emerged, touting microvascular dysfunction as a promising therapeutic target in this burgeoning condition that predominantly impacts older women [189, 190]. This has led to the hypothesis that risk factor conditions (age, obesity, dysglycemia, hyperlipidemia), including loss of estrogen, promote a pro-inflammatory, pro-oxidative state, rendering the microvasculature vulnerable [191, 192]. Thus, while “microvascular dysfunction” may present itself in specific end-organs like the myocardium (i.e. coronary microvascular dysfunction, and associated ischemia, structural remodeling, systolic/diastolic dysfunction), this conceptual framework suggests that microvascular dysfunction is likely systemic in nature.

The assessment of “microvascular function” has therefore taken a broad approach in recent years, ranging from circulating biomarkers (sICAM-1 [soluble intercellular adhesion molecule-1], sVCAM-1 [soluble vascular adhesion molecule-1], sE-selectin [soluble E-selectin], and vWF [von Willebrand

factor]), structural imaging approaches like optical coherence tomography [193], and darkfield microscopy [194–196] to limb reperfusion measurements following a brief period of tissue ischemia (i.e. reactive hyperemia) [197, 198]. While each of these endpoints have been studied in the context of specific cardiovascular and/or metabolic diseases, unlike studies evaluating macrovascular endothelial function, population studies exploring sex and the influence of healthy aging on microvascular endpoints remain limited. More work is therefore needed to fully elucidate the impact of age and sex on microvascular dysfunction, along with specific mechanisms contributing to its prevalence.

### Neurovascular Control

Accumulating evidence suggests that both sex and age influence autonomic neural control of vascular tone. For example, the incidence of orthostatic intolerance is much higher in young women than young men, related to an apparent attenuation of peripheral vasoconstrictor responsiveness to sympathetic activity [199–201]. Where a significant relationship exists between sympathetic nerve activity and total peripheral resistance in young men, this relationship is absent in young women [202], attributable to greater  $\beta$ -adrenergic-mediated vasodilation in young women [203, 204]. Indeed, the relationship between sympathetic nerve activity and total peripheral resistance is restored in young women via systemic  $\beta$ -adrenoreceptor blockade [204]. After ~40 years of age, however, the autonomic nervous system plays a much more dominant role in the control of blood pressure [205], a response largely attributable to a reduction in  $\beta$ 2-adrenergic receptor ( $\beta$ 2AR)-mediated vasodilation [203, 206, 207]. While the exact mechanism for this finding remains incompletely understood, reduced NO bioavailability has been implicated [207]. Moreover, aged postmenopausal women have greater vasoconstrictor responses to norepinephrine [203], which may be related to greater sympathetic transduction of sympathetic nerve activity [208]. It is interesting to consider these findings in the context of popular cardiovascular therapeutics, particularly beta- and alpha-blockers. Caution may therefore be warranted as we promote certain classes of drugs that have worked well in male-dominated conditions like heart failure with reduced ejection fraction (HFrEF) to more female-dominant conditions like HFpEF.

### Conclusions

Taken together, there is clear evidence that both age and sex influence the cardiovascular system. Sex-specific cardiomyocyte loss, an increase in extracellular matrix, and myocellular hypertrophy work in tandem in the presence of CVD risk factors and/or overt CVD to promote unique patterns of LV



remodeling in women and men. In addition, age- and sex-specific vascular stiffening is also well established, driven by changes in endothelial dysfunction, elastin–collagen content, microvascular function, and neurohormonal signaling. Together, these changes in LV chamber geometry and morphology, coupled with heightened vascular stiffness, appear to drive both age-related increases in systolic function and declines in diastolic function, particularly in postmenopausal women. Estrogen is indeed implicated as an important mediator of the aforementioned changes, given that it is a direct vasodilator, promotes nitric oxide excretion, and impacts myocellular  $\text{Ca}^{2+}$  handling, mitochondrial energy production, and oxidative stress. The culmination of these sex-specific cardiac and vascular changes across the lifespan may provide key insight into heart failure development, particularly of the preserved ejection fraction variety. While knowledge gaps remain, as outlined herein, the collective insight currently available offers great promise for future preventive strategies and therapeutic approaches.

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## Compliance with Ethical Standards

**Conflict of Interest** C.N.B.M receives funding from Abbott Diagnostics, Sanofi (paid through CSMC) and serves as Board of Directors for iRhythm. Andrew Oneglia and Dr. Michael Nelson declare that they have no conflict of interest. Dr. Bairey Merz reports personal fees from iRhythm, personal fees from Med Intelligence, personal fees from Bayer Advisory Board, grants from California Institute for Precision Medicine, grants from CDMRP Department of Defense, grants from NHLBI subcontract to Research Triangle Institute (RTI) International, grants from the NIH, and grants from Sanofi, outside the submitted work.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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