Premenopausal women experience lower rates of cardiovascular disease (CVD) compared with age-matched men; a benefit thought to be mediated, at least in part, by the female sex hormone estrogen.1,2 Consistent with that notion, despite a lower CVD in younger women, the rate of CVD development and mortality in women after menopause exceeds that of men.2,3 These findings underscore the critical need to understand both baseline differences in cardiovascular function and responses to pathological cardiac insults between the sexes.4 Indeed, major funding agencies in the United States, Canada, and Europe have emphasized the inclusion of both sexes; however, women and female animals remain vastly under-represented in all stages of CVD research.5–8 Until recently, consideration of both sexes was not required in clinical and preclinical studies that focus on CVD development, animal models are essential tools and should be useful in the development of therapeutics. This review will focus on describing the cardiovascular sexual dimorphisms that exist both physiologically and in common animal models of CVD. (Circ Res. 2016;118:1294-1312. DOI: 10.1161/CIRCRESAHA.116.307509.)

Key Words: cardiovascular diseases ■ estrogens ■ gonadal steroid hormones ■ heart failure ■ sex characteristics

**Abstract:** Nearly one-third of deaths in the United States are caused by cardiovascular disease (CVD) each year. In the past, CVD was thought to mainly affect men, leading to the exclusion of women and female animals from clinical studies and preclinical research. In light of sexual dimorphisms in CVD, a need exists to examine baseline cardiac differences in humans and the animals used to model CVD. In humans, sex differences are apparent at every level of cardiovascular physiology from action potential duration and mitochondrial energetics to cardiac myocyte and whole-heart contractile function. Biological sex is an important modifier of the development of CVD with younger women generally being protected, but this cardioprotection is lost later in life, suggesting a role for estrogen. Although endogenous estrogen is most likely a mediator of the observed functional differences in both health and disease, the signaling mechanisms involved are complex and are not yet fully understood. To investigate how sex modulates CVD development, animal models are essential tools and should be useful in the development of therapeutics. This review will focus on describing the cardiovascular sexual dimorphisms that exist both physiologically and in common animal models of CVD.

**Sexual Dimorphisms in Human and Rodent Cardiovascular Physiology at Baseline**

Although baseline characteristics of cardiac function in healthy men and women differ in terms of heart rate, left ventricular (LV) ejection fraction, and stroke volume, cardiac functional advantages in healthy men compared with women have been debated in the literature for many years.11,12 Higher LV ejection fraction and stroke volume in men develop after adolescence, suggesting a role for sex hormones in the understanding of sex differences in both cardiac health and pathology, we will review molecular and functional differences between healthy men and women and will relate these findings to healthy rodents used in CVD research. Although both male and female sex hormones modulate cardiac function, we focus on the genomic effects of estrogen as the major mediator of sex differences. Finally, we present molecular and functional characteristics of animal models of CVD, emphasizing sex differences in their phenotypes.
Cardiac Contractility and Ion Channels

Consistent with cardiac functional differences observed in humans, cardiac myocytes isolated from male rodents contract more strongly and rapidly than female cardiac myocytes, a difference that is reduced with age. In addition, relaxation rates differ between the sexes. Although conflicting results have been reported, when differences are observed, it is the female cardiac myocyte that relaxes more slowly. Sex differences in cardiac ion channel expression and function have been a focus of many studies and are implicated in the cellular basis for these differences in cardiac contractility. For example, the amplitude and frequency of calcium sparks produced by the release of calcium from the sarcoplasmic reticulum (SR) are higher in male rat cardiac myocytes. Detailed electrophysiological measurements of action potentials in individual cardiac myocytes have revealed specific roles of many different types of ion channels that may mediate differences in conductivity and contraction in the hearts of men and women, as well as animal models of CVD.

Expression and activity of sodium, calcium, and potassium channels dramatically affect the contractility of the heart through modulation of components of the action potential (Figure 1); the sequence and duration of ionic movement in the cardiac myocyte dictate the strength and frequency of contraction. When a sufficiently depolarizing current reaches the cardiac myocyte, sodium channels open to rapidly depolarize the cell, which promotes opening of voltage-gated L-type calcium channels. Increased calcium concentrations in the cardiac myocyte prolong repolarization and refractory durations and promote contraction through the interaction between calcium and troponin. The plateau phase, during which contraction takes place, is achieved through continued slow inward movement of calcium through L-type calcium channels and flow of potassium out of the cell through slow delayed rectifier channels that begin repolarizing the membrane potential. The cardiac myocyte returns to the resting membrane potential by the removal of calcium from the sarcoplasm and continued flow of potassium out of the cell through potassium channels. Importantly, this plateau and slow repolarization produces a refractory period in which the cell cannot be depolarized, thus preventing tetanus.

The mechanisms mediating these differences in the healthy heart have not been fully elucidated. The need for experimental manipulation of potential modulators requires the use of animal models to study on molecular, cellular, and systemic levels and the complicated interactions among sex, cardiac myocytes, and the cardiovascular system. As in humans, sex differences in baseline cardiovascular function are observed in many experimental rodents. In light of the dramatic effects sex has on cardiovascular function in humans, the National Institutes of Health has called for the inclusion of both male and female animals in preclinical CVD research.

The question of whether cardiovascular function/dysfunction in animals translates to human cardiovascular physiology has been a challenge in research for nearly a century. Basic cellular and functional differences in some experimental models are similar to those of humans, making the use of animals to model human disease possible. For example, the development of the mouse heart remarkably recapitulates that of the human heart. Notably, the role of sex hormones, particularly estrogen, in cardiovascular function is also similar in animals compared with humans. The use of animal models has, therefore, allowed elucidation of specific effects of sex hormones on cardiac function that include direct and indirect modulation of contractility, ion channel expression and function, reactive oxygen species production, and substrate use, among others.
Each of these currents, particularly those mediated by calcium and potassium channels, exhibits significant sexual dimorphisms. Female hearts, for example, exhibit a longer repolarization phase mediated by potassium channels than male hearts (Figure 1), a characteristic that develops shortly after puberty and leaves women more prone to cardiac arrhythmias than men.\textsuperscript{26,27} Interestingly, tissue samples from healthy hearts of female donors reveal lower levels of potassium channel proteins than male donor hearts, including the Kv1.4, Kv channel–interacting protein 2, sulfonylurea receptor 2, and human ether-a-go-go subunits that are responsible for cardiac myocyte repolarization\textsuperscript{28}; lower levels of proteins that contribute to \(I_{\text{Ks}}\) and \(I_{\text{Kr}}\) prolong the QT interval.\textsuperscript{29} In women, longer repolarization during normal sinus rhythm is likely attributable to a higher contribution of slow rectifying potassium channels based on the morphology of the action potential.\textsuperscript{24} Similar to humans, action potential duration in female rodents is longer than in male rodents caused by a longer repolarization segment. However, mice and rats do not exhibit a plateau phase and rapidly repolarize without the delayed rectifiers \(I_{\text{Kr}}\) and \(I_{\text{Ks}}\) observed in human hearts (Figure 2A and 2B).\textsuperscript{30,31}

The movement of calcium into the cell during depolarization of the cardiac myocyte activates the release of calcium from the SR in both humans and rodents, thus producing higher intracellular calcium and initiating contraction. Although this process of electrochemical signaling through the heart is recognized as the process by which mammalian hearts contract, excitation–contraction coupling and the mechanisms that mediate it vary among species and between the sexes. Expression of several types of pumps and channels mediates the movement of calcium, including the sodium–calcium exchanger (NCX), SR calcium ATPase, sarcolemmal calcium ATPase, and a calcium uniporter expressed on the membranes of mitochondria. SR calcium-ATPase pumps are responsible for \(\approx 70\%\) of calcium removal to produce relaxation in humans.\textsuperscript{32} By contrast, in rats and mice, >90\% of calcium removal is mediated by the SR calcium ATPase because of higher expression of the transporter.\textsuperscript{33} Interestingly, in female rat hearts, diastolic concentrations of calcium inside the SR and the rate of reuptake after contraction are lower than in male rat hearts, whereas in mice, sex differences are not observed.\textsuperscript{31} Other studies in rats have revealed weaker and slower contractions in isolated female cardiac myocytes that could be explained by sex differences in calcium stored in the SR rather than differences in influx mediated by NCX or altered myofilament calcium sensitivity.\textsuperscript{21} Despite evidence in rodents supporting a role for SR-mediating calcium flux as a mechanism responsible for differences in contractility, these studies have not been fully supported by human data.

Significant differences also arise between human and animal depolarizing currents mediated by calcium and sodium channels. In female dogs, for example, the density of depolarizing calcium currents mediated by L-type calcium channels is higher than in males, consistent with a more rapid depolarizing calcium currents mediated by L-type calcium channels.\textsuperscript{34} Indeed, estrogen receptor (ER)–deficient male mice also exhibit higher expression of L-type calcium channels.\textsuperscript{35} Although Cav1.2, which mediates calcium entry into the cardiac myocyte and plays an important role in depolarization, is reduced by estrogen, the NCX, which is involved in repolarization, is increased by estrogen; ovariectomy reversed this expression pattern in female rats,\textsuperscript{36} which is significant in light of prolonged repolarization in women. NCX mediates calcium influx and maintains the action potential plateau.\textsuperscript{37} In rats, calcium concentrations in the cytosol are predominantly mediated by the SR rather than by NCX as in humans.\textsuperscript{31} Currents mediated by L-type calcium channels are rapidly inhibited by exposure to estrogen or testosterone in rat and guinea pig cardiac myocytes.\textsuperscript{38–40} Opposing effects on ion currents by steroid hormones are emphasized by the decrease in potassium currents by estrogen, and the increase in potassium currents by testosterone could account for longer

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Action potential waveform in adult human (A) and mouse ventricular cardiac myocytes (B). Currents \((i)\) contributing to each component of the action potential are shown below. \(I_{\text{Na}}\) indicates L-type slow inward calcium current; \(I_{\text{Kr}}\), rapid delayed potassium rectifier; \(I_{\text{Ks}}\), slow delayed potassium rectifier; \(I_{\text{Na,K}}\), sodium current; \(I_{\text{CaL}}\), fast transient outward potassium current; and \(I_{\text{NCX}}\), slow transient outward potassium current. Reprinted from Nerbonne\textsuperscript{30} with permission of the publisher. Copyright ©2003, Springer.}
\end{figure}
repolarization durations in the hearts of women.\textsuperscript{38-40} Despite striking differences between the action potential segment durations and heart rates, mechanisms mediating the ionic currents are conserved and sex differences observed in humans are also present in rodents. Thus, if one were interested in mechanisms mediating sex differences, rodent models are clinically relevant.

**Molecular Biology of the Heart and the Cardiac Myocyte**

Sex differences in cardiac gene expression are present in both humans and rodents. In humans (<40 years old) and mice (2 months old), expression of a similar number of cardiac genes is different between men and women, many of which are expressed on sex chromosomes, a mechanism requiring more study.\textsuperscript{41,42} Cardiac genes expressed on autosomal chromosomes also differ between the sexes, and in mice, these differences seem to be independent of the estrous cycle.\textsuperscript{41} Notably, several GeneOntology categories that are different between the sexes are similar when humans and mice are compared, including genes mediating chemotaxis and inflammation. However, in humans, these categories were over-represented in men, whereas in mice, enriched genes were primarily observed in the hearts of women.\textsuperscript{41}

Sexual dimorphisms that are species specific are also observed in expression of genes encoding contractile proteins. In the human heart, cardiac myosin expression is dominated by $\beta$-myosin heavy chain ($\beta$MyHC), with $>90\%$ of total MYHC composed of $\beta$MyHC, and is higher in the atria and ventricles of healthy women compared with men.\textsuperscript{38,43} By contrast, in mice and rats, expression of $\alpha$MyHC dominates the ventricles, whereas in the hearts of larger animal models with slower heart rates, such as dogs and rabbits, MYHC isoform distribution more closely resembles that of humans.\textsuperscript{31,45} On average, the healthy murine heart is composed of $>95\%$ $\alpha$MyHC.\textsuperscript{46} However, as in humans, rodent hearts exhibit sex differences in MYHC isoforms; female rat hearts, for example, express higher $\alpha$MyHC and lower $\beta$MyHC compared with male rat hearts.\textsuperscript{47} In fact, the absence of estrogen in women reduces the levels of $\alpha$MyHC,\textsuperscript{48} again supporting a molecular advantage in the healthy murine heart composed of $>95\%$ $\alpha$MyHC.\textsuperscript{46} Interestingly, substrate use in the absence of increased workload differs between men and women. In a small study of men and women, cardiac use of glucose was significantly higher in men, suggesting that substrate preference may play a role in women being affected more negatively by ischemic disease, such as myocardial infarction (MI).\textsuperscript{34}

Baseline mitochondrial function in many female animal models is consistent with cardioprotection mediated by healthy cardiac metabolism. Older female rat hearts have higher oxidative phosphorylation capacity compared with age-matched males, whereas this capacity is nearly equal in younger rats of either sex.\textsuperscript{55,56} In addition, lower production of reactive oxygen species is observed in young female rats, which is promoted by higher level of aldehyde dehydrogenase expression and activity and reduced production of reactive oxygen species by $\alpha$-ketoglutarate dehydrogenase, supporting observations in women, suggesting that the hearts of women have less-oxidative damage over a lifetime.\textsuperscript{7,58} Young adult female Fischer 344 rat hearts also exhibit higher expression of nuclear genes with a role in $\beta$-oxidation of fatty acids compared with the hearts of young men,\textsuperscript{56} similar to humans where the hearts of premenopausal women favor fatty acid substrates over glucose compared with age-matched men. In fact, estrogen decreases the expression of glucose transporter type 4 via increased expression of nitric oxide (NO) synthase (NOS) in both humans and rats,\textsuperscript{55,59} thereby forcing the use of fatty acids under normoxic conditions. Much remains to be learned about how sex influences mitochondrial number and function in the heart; interactions among $\approx$1500 genes contribute to normal function.\textsuperscript{60} At a basic level, however, rodents exhibit similar cardiac metabolic characteristics to humans.

**Hormones Implicated in Sexually Dimorphic Cardiac Function**

In humans, it has long been thought that the female sex hormone estrogen and signaling through ERs expressed in the vasculature and the heart are primarily responsible for the cardiac protection experienced by premenopausal women compared with age-matched men.\textsuperscript{61} Estradiol is synthesized through the aromatization of testosterone and interacts with 2 main receptors that are localized in the nucleus, in the cytoplasm, at the plasma membrane, and on mitochondria: ER$\alpha$ and ER$\beta$. ERs are primarily localized in the nucleus where they bind to estrogen response elements or to other transcription factors and regulate transcription of genes mediating cell growth, contractility, apoptosis, and energy substrate use.\textsuperscript{18,62} A third protein, G protein–coupled receptor (GPR30 [G protein-coupled receptor 30] or GPER [G protein-coupled estrogen receptor]), has been implicated in mediating rapid, nongenomic estrogen signaling independently of the canonical ERs.\textsuperscript{63-65} However, studies suggest that GPER plays a role in estrogen signaling by regulating the expression of an extranuclear ER$\alpha$ isoform (ER$\alpha$36), not by GPER itself binding to estrogen.\textsuperscript{66} Because of multiple conflicting findings on GPER’s ER status, this review will focus on more classical estrogen signaling pathways mediated through either ER$\alpha$ or ER$\beta$. However, when pathology is introduced, a switch in glucose use occurs, to an anaerobic process that allows ATP production even under conditions of low oxygen, such as ischemia.\textsuperscript{53}

Alterations of cardiac bioenergetics are indicators of physiological or pathological responses to workload. Under normal conditions, the heart uses fatty acids as an energy substrate.
An abundance of studies in premenopausal and postmenopausal women, including those receiving hormone replacement therapy (HRT), implicate estrogen in altering cardiovascular function. The Women’s Health Initiative clinical trial suggested that HRT initiation in postmenopausal women is associated with increased CVD; the trial was halted early (at 5.2 years) because of concerns about adverse events in the heart, pulmonary emboli, and in cancer. In addition, results from the Heart and Estrogen/Progestin Replacement Study revealed that HRT in postmenopausal women with existing CVD did not improve, and again, a significant trend toward worse cardiac outcomes was apparent. However, most of these women had been postmenopausal for significant periods of time and with additional health conditions, complicating interpretation of the results. Recently, a randomized study involving 727 women within 3 years of their last menses revealed that earlier hormone replacement improves some aspects of CVD risk, and participants receiving HRT did not differ in their rates of atherosclerosis progression. Other steroid hormones, such as progesterone and testosterone, that are expressed in both men and women and are also implicated in modulating cardiac function and disease phenotype exhibit cyclic concentrations in serum and decrease with age.

The attention given to HRT initiated interest in the potential therapeutic abilities of products that contain phytoestrogens, such as soy, that have the ability to initiate estrogenic signaling by binding ERα or ERβ. However, after several clinical trials with postmenopausal women, the cardiovascular benefits of soy supplementation remained controversial and the American Heart Association reversed its endorsement of soy products for this purpose. In addition, phytoestrogens present in standard laboratory rodent chow, such as genistein, directly alter contractility and inhibit tyrosine kinase activation in cardiac myocytes, which should be considered when performing animal experiments.

Clinical studies that identified sex differences in CVD where the findings are abrogated in postmenopausal women have motivated a large number of studies that manipulate levels of sex hormones, particularly estrogen, in experimental animals. Comparisons between women and female experimental animals are challenging because of significant differences in estrous cycle duration and serum estrogen levels. In mice, for example, the estrous cycle varies from 2 to 7 days, and decreased cycling is observed in older female mice (aged >13 months). Female rodents do not go through a significant decline in estrogen levels between young C57Bl6 mice and older (aged >13 months). Adult rat ventricular myocytes (ARVMs) isolated from male and female rats were treated with physiological doses of estradiol. Alterations in the phosphorylation of 39 RTKs and 46 intracellular signaling molecules were measured by comparing lysates of estrogen-vehicle-treated ARVMs. A consistent observation among experiments is that the basal levels of RTK phosphorylation are significantly higher (≈4.5-fold) in untreated ARVMs isolated from female rats compared with male rats (n=2–3 separate isolations per sex) (Figure 3A). In response to estradiol treatment, phosphorylation of many RTKs was reduced in female cells compared with male cells (Figure 3B). When considered

**Figure 3.** Phosphorylation of receptor tyrosine kinases (RTKs) in adult rat ventricular myocytes (ARVMs) isolated from male and female rats. A. Baseline phosphorylation levels of RTKs in male and female ARVMs. B. Phosphorylation levels of RTKs in female and male ARVMs treated with 300 nmol/L estradiol, relative to vehicle treated. Light gray bars, female; dark gray bars, male. A. n=2 pooled preparations, 2 to 3 rats per preparation. B. n=3 pooled preparations, 1 rat per preparation. FGFR indicates fibroblast growth factor receptor; Fit-3, Fms-like tyrosine kinase 3; IGF-IR, insulin-like growth factor 1 receptor; PDGF, platelet-derived growth factor; and VEGFR, vascular endothelial growth factor receptor.

**Intracellular Signaling in the Cardiac Myocyte of Male and Female Rats**

Data in our laboratory suggest a sexually dimorphic role for estrogen in the modulation of signaling activity in cardiac myocytes. Activation of receptor tyrosine kinases (RTKs), for example, has been implicated in several important cardiac functions, including cardiac myocyte hypertrophy, contractility, and vascular growth. Adult rat ventricular myocytes (ARVMs) isolated from male and female rats were treated with physiological doses of estradiol. Alterations in the phosphorylation of 39 RTKs and 46 intracellular signaling molecules were measured by comparing lysates of estrogen-vehicle-treated ARVMs. A consistent observation among experiments is that the basal levels of RTK phosphorylation are significantly higher (≈4.5-fold) in untreated ARVMs isolated from female rats compared with male rats (n=2–3 separate isolations per sex) (Figure 3A). In response to estradiol treatment, phosphorylation of many RTKs was reduced in female cells compared with male cells (Figure 3B). When considered

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collectively, RTK phosphorylation with estradiol treatment in female ARVMs was reduced by 61.8% but increased 55.9% in male cells, indicating that estrogen exhibits opposing effects on RTK phosphorylation in male and female cardiac myocytes. Interestingly, addition of estradiol to female ARVMs normalized the difference between activation of intracellular kinases between male and female vehicle-treated cells; 3.9-fold higher activation in female cells was reduced to <5% with the addition of estradiol. The addition of physiological doses of estradiol did not affect mRNA levels of 7 of the most highly inhibited RTKs (data not shown), suggesting that transcriptional or post-transcriptional regulation by estradiol does not significantly affect the data. These data are supported by inhibition of growth factor signaling by estrogenic compounds that signal through ERs and require further investigation but suggest a possible novel mechanism by which estrogen modulates signaling in cardiac myocytes.81

Animal Models of CVD

Hypertension

More than 95% of hypertension cases are classified as essential hypertension without a single cause.82 Sex differences in blood pressure, such as many cardiovascular features, originate during adolescence with persistently higher systolic and diastolic pressures observed in men.83,84 In addition, sex differences are also observed in pulmonary hypertension, which progressively leads to right heart failure (HF) with women being at greater risk than men.85 The review by Mair et al85 provides an in-depth account for these differences as it is beyond the scope of this article to discuss pulmonary hypertension. The key organ for long-term control of blood pressure and body fluid volume is the kidney.56,87 According to the renal body fluid feedback concept, chronic increases in arterial pressure occur as the result of abnormalities in the relationship between renal perfusion pressure and sodium excretion. That is, in order for long-term increases in arterial pressure to occur, a reduction in the kidneys’ capacity to excrete sodium and water must be present. A common defect that has been found in all forms of hypertension examined to date, including genetic and experimental animal models and human essential hypertension, is a rightward shift (toward increased blood pressure) in the chronic renal pressure–natriuresis relationship. The renin–angiotensin system and other hormones, including sex steroid hormones, modulate baseline sexual dimorphisms in blood pressure.88,89 In men, plasma renin activity is ≈27% higher than in women, regardless of age, but renin activity increases in postmenopausal women, an effect that may be mediated by lower estrogen or influenced by increases in testosterone.90 In addition, dietary intake of salt can dramatically alter blood pressure, especially in blacks. It is now recognized that blood pressure is salt sensitive in ≤30% to 50% of hypertensive individuals.88 Interestingly, blood pressure in premenopausal women is relatively unaffected by sodium intake, but blood pressure becomes more salt sensitive with the onset of menopause in many women.91

Spontaneously Hypertensive Rat Model

In 1963, a male rat with essential hypertension that was 25 mm Hg higher than normal was identified and bred to produce a line of spontaneously hypertensive rats (SHRs).92 Although this is a genetic model of hypertension, the underlying genetic variations responsible for disease development are extremely complex as quantitative trait locus mapping experiments have identified multiple genes that may be associated with essential hypertension.93,94 As male and female SHR animals mature, both sexes exhibit increased systolic blood pressure; however, by 12 weeks of age, this increase is significantly higher in the male animals compared with female animals, similar to humans.95 This sexual dimorphism persists through adulthood; male SHRs are more hypertensive than female SHRs until after female rats stop estrus cycling (aged 10–12 months), when, by 16 months of age, blood pressure is higher in female rats than male rats.96 Male SHRs also develop signs of HF by 24 months, but female animals do not develop the ventricular stiffness or dilation that is observed in the male animals.96 Sexual dimorphisms are also observed at the level of the cardiac myocyte as SHR LV myocyte diastolic and systolic sarcomere dynamics were reduced compared with normotensive controls; this observation was more pronounced in male SHR myocytes.97 The progress of disease and sexual dimorphisms are similar to those observed in men and women, making this model particularly useful.98

Sex hormones seem to play an important role in mediating these observed sex differences. When young male and female SHRs were castrated or ovariectomized to deplete endogenous sex hormones, castrated male SHRs exhibited reduced blood pressure similar to that of female SHRs.95 In this study, ovariectomy alone did not affect blood pressure. However, supplementing ovariectomized female rats with testosterone increased blood pressure by 10%, suggesting that androgens may mediate the observed sex difference between SHRs,95 and that female sex hormones are not protective for hypertension in the female SHR.96,100 Interestingly, sex differences are also influenced by the renin–angiotensin system. When male and female SHRs, both intact and gonadectomized, were treated with an angiotensin-converting enzyme inhibitor, sex differences were abrogated as reduced blood pressure was most significant in male rats and ovariectomized female rats supplemented with testosterone after treatment.101 The renin–angiotensin system also mediates hypertension in aged SHRs; treatment of 16-month-old male and female SHRs with the angiotensin receptor antagonist, losartan, decreased blood pressure in both sexes.102 However, the decreases were greater in male SHRs, suggesting that renin–angiotensin system may be more important in aging male SHRs than female SHRs for maintaining blood pressure.

Dahl Salt-Sensitive Rat Model

Increases in dietary sodium can lead to dramatic increases in blood pressure. Men that have developed salt-sensitive hypertension are at greater risk of early death than women although women are more salt sensitive than men in terms of blood pressure.103 The Dahl salt-sensitive (DSS) rat, a genetic model of hypertension induced by feeding the animals a high-sodium diet, demonstrates sex differences similar to those observed in
men and women. Before the addition of a high salt diet, female salt-sensitive rats have significantly lower basal systolic blood pressure compared with male rats. Female DSS rats have also been shown to display significantly lower systolic blood pressure than male rats after being fed a high salt diet for 3 weeks. In this same study, hypertension induced by 4 weeks of a high salt diet in both male and female DSS rats resulted in 50% mortality in male rats only. Unlike the SHR model, female sex hormones contribute to protection against hypertension as ovariectomy of female DSS rats increased basal blood pressure compared with intact female rats. In addition, ovariectomized female DSS rats fed a high salt diet developed hypertension in a manner that was not significantly different from male rats. However, when dietary sodium was decreased to normal levels, blood pressure in the male and intact female DSS rats decreased, whereas in the ovariectomized female DSS animals, it remained unchanged, suggesting that removing the female sex hormones predisposes the DSS female rats to develop hypertension, independent of sodium intake. Testosterone also seems to have a role in this process as castration of male DSS rats fed a high salt diet attenuated the development of both hypertension and the increased expression of renal angiotensinogen. In addition, castrated DSS male rats fed a high salt diet supplemented with testosterone had elevated blood pressure and increased renal injury. Future studies are necessary to completely understand the role of male and female hormones during development of salt-sensitive hypertension.

### Table 1. Overview of Sex Differences in Animal Models of Hypertension

<table>
<thead>
<tr>
<th>Model</th>
<th>Age</th>
<th>Treatment</th>
<th>Reported in Male Rats</th>
<th>Reported in Female Rats</th>
<th>Molecules/Pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>12–20 wk</td>
<td>NA</td>
<td>Higher systolic and mean arterial blood pressure</td>
<td>Testosterone; RAS signaling</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>9–12 wk</td>
<td>NA</td>
<td>Isolated cardiac myocytes displayed reduced diastolic and systolic sarcomere dynamics</td>
<td>Not addressed</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>3–30 mo</td>
<td>NA</td>
<td>Progressed to failure by 15 mo</td>
<td>Less hypertensive until 18 mo; better mortality rates</td>
<td>Not addressed</td>
</tr>
<tr>
<td>SHR</td>
<td>16 mo</td>
<td>Losartan (30 mg/kg per day): 3 wk</td>
<td>Displayed an enhanced depressor response after angiotensin receptor antagonism</td>
<td>RAS</td>
<td></td>
</tr>
<tr>
<td>DSS</td>
<td>4–5 wk</td>
<td>Low salt (0.3%) or high salt (8%); diet: 3 wk</td>
<td>Higher blood pressure and increased mortality in response to high salt diet</td>
<td>Lower basal systolic blood pressure</td>
<td>Vasodilatory prostaglandins that were higher in female rats</td>
</tr>
<tr>
<td>DSS</td>
<td>17 wk</td>
<td>Low salt (0.28%) or high salt (8%); diet: 4 wk</td>
<td>Expresses higher levels of renal angiotensinogen mRNA and protein in response to high salt diet; this response was attenuated by castration</td>
<td>Testosterone</td>
<td></td>
</tr>
<tr>
<td>DSS</td>
<td>14 wk</td>
<td>Low salt (0.4%) or high salt (4%); diet: 2 wk</td>
<td>O VX rats displayed increased blood pressure at baseline and in response to high salt diet</td>
<td>Estrogen potentially regulating NO</td>
<td></td>
</tr>
<tr>
<td>DSS</td>
<td>8–9 wk</td>
<td>Varying salt diets: 0.15% (7 d), 1% (14 d), 4% (14 d), 8% (14 d)</td>
<td>Salt-dependent hypertension was greater</td>
<td>O VX rats developed hypertension similar to male rats, but their blood pressure did not normalize on return to normal diet</td>
<td>Estrogen</td>
</tr>
<tr>
<td>L-NAME treatment—rats</td>
<td>13–14 wk</td>
<td>L-NAME (75 mg/100 mL drinking water): 5 wk</td>
<td>Blood pressure in male rats was higher after treatment; this sex difference was abolished on castration of male rats</td>
<td>O VX had no effect</td>
<td>Testosterone</td>
</tr>
<tr>
<td>L-NAME treatment—rats</td>
<td>12 wk</td>
<td>L-NAME (50 mg/100 mL drinking water): 4 wk</td>
<td>No difference</td>
<td>No difference</td>
<td>NA</td>
</tr>
<tr>
<td>L-NAME treatment—rats</td>
<td>7 wk</td>
<td>L-NAME (20 mg/100 mL drinking water); withdrawal observed for 7 wk</td>
<td>Developed more severe and rapid hypertension; took longer to respond to withdrawal of treatment</td>
<td>Not addressed</td>
<td></td>
</tr>
</tbody>
</table>

DSS indicates Dahl salt-sensitive; L-NAME, Nω-nitro-l-arginine methyl ester; NA, not applicable; NO, nitric oxide; RAS, renin-angiotensin system; and SHR, spontaneously hypertensive rat.
with female rats. In addition, castration of male rats attenuated the development of hypertension, whereas ovariectomy of L-NAME–treated female rats did not affect blood pressure. These results suggest that estrogen is not mediating the protective effect observed in female rats with L-NAME. Although these results seem similar to other models of hypertension discussed earlier, there are contradictory reports on the sexually dimorphic response to L-NAME treatment. Other groups have observed that female animals actually develop more hypertension than male animals or report that there is no difference in blood pressure in response to NOS inhibition between the sexes. These conflicting data could be because of the type of L-NAME treatment as each of these studies used a different dose and length of treatment to induce hypertension. Even with these discrepancies, sex differences with respect to hypertension development after L-NAME treatment are apparent in other rat models, such as SHR animals and normotensive Sprague Dawley rats. Although blood pressure in SHR male rats is greater than female rats at baseline, female rats exhibited a greater increase in blood pressure after L-NAME treatment, suggesting that female SHRs are more sensitive to NOS inhibition than male SHRs. However, these data are consistent with studies showing that estradiol increases NOS III and NOS I synthesis.

The mechanisms responsible for hypertension are multifactorial with a combination of genetic and environmental influences; therefore, no one animal model will completely mimic human disease development. Although the SHR model is a commonly used animal model of essential hypertension, the DSS rat is a model of salt-sensitive hypertension observed in humans. However, SHRs are not prone to strokes or vascular thrombosis, and high salt diets cause significant renal injury and mortality within 4 to 6 weeks in DSS rats. Rats also do not develop signs of atherosclerosis. However, all of the discussed rodent models demonstrate increases in blood pressure in a relatively rapid and reproducible manner, providing reliable experimental systems for hypertension induced by independent mechanisms. Overall, these animal models mirror sex differences observed in humans with women developing less hypertension than men, aging confounding the hypertension in women, and estrogen signaling playing a critical role in mediating certain aspects of this protection.

**MI and Ischemic Injury**

Each year, >600,000 Americans will experience a new MI event and ≈40% of these cases will progress to HF. Sex differences with respect to MI and HF are observed, with women seemingly protected in that they develop the disease later in life compared with men. However, men and women often present different disease symptoms, and young women hospitalized for acute MI actually have worse outcomes than their male counterparts. In addition, after menopause, the prognosis for women with MI is significantly worse than that for age-matched men. Cardiac remodeling in response to ischemic injury exhibits hallmarks of cardiac myocyte death, inflammatory cell infiltrations, and the development of fibrosis within the injured area in both men and women. Remodeling also occurs in the surrounding healthy tissue, including cardiac myocyte hypertrophy and altered ion channel expression that causes arrhythmias such that, in humans, the degree of remodeling negatively correlates with mortality. In addition, trends of decreased SR calcium ATPase, phospholamban, and ryanodine receptors are observed, indicating reduced calcium transients and decreased contractility in human HF. Similar to humans, animal models also display cardiac remodeling and sexually dimorphic characteristics with respect to ischemic injury development, severity, and response to reperfusion (Table 2).

### Models of Myocardial Injury Induced by Coronary Artery Ligation

The complex nature of MI and HF has made developing a single animal model difficult. However, ligation of the left coronary artery in a variety of different rodent species recapitulates much of what is observed in human patients. Previous studies have used rodents to investigate sex differences observed both in the initial response to MI and the development of HF. For example, the rate of cardiac rupture and mortality within the first week after MI was greater in male mice compared with female mice, regardless of infarct size. In addition, immediately after MI, the hearts of male mice have increased neutrophil infiltration, damage to the interstitial collagen network, and matrix metalloproteinase activity compared with their female counterparts. Furthermore, despite infarct size being equal at intermediate time points, during the chronic phase 12 weeks after MI, male mice displayed worse cardiac function, more cardiac myocyte hypertrophy, and increased ventricular dilation compared with female counterparts. However, female mice were able to maintain contractile function over time, whereas male mice displayed progressive declines in contractile function associated with maladaptive cardiac remodeling. The cardiac phenotypes of mice with experimentally induced MI are consistent with humans with MI in that the hearts of women with MI exhibit lower rates of myocardial cell death and progress to HF more slowly than men. In addition, as in humans, alterations in potassium- and calcium-channel expression and currents that prolong the repolarization segment led to arrhythmogenicity in post-MI rats, and estrogen reduces post-MI arrhythmias associated with these ionic changes in mice.

Rats with experimental MI show variable and contrasting results compared with what has been reported in mice. In response to MI, male and female rats developed similar size infarcts and did not exhibit differences in contractile function 6 weeks after injury. However, 6 weeks after MI, male animals displayed restriction of LV filling, as well as greater increase in LV posterior wall thickness and myocardial diameter compared with female animals. In contrast, Chen et al demonstrated that 4 weeks after MI, female rats displayed more dilation than male rats. Analysis of isolated cardiac myocytes from infarcted rat hearts also exhibited no differences in morphometrics in response to MI between sexes. In a similar study that analyzed scar composition 4 weeks post MI, male rats developed larger MI scars than female rats, but the overall structural composition of the scars was not different between sexes. Aged male and female rats displayed similar patterns of overall LV remodeling, but differences were apparent in regional cardiomyocyte hypertrophy and arteriole expansion.
4 weeks post MI. Together, these studies demonstrate that sex differences do exist in rodents with experimental MI, but inconsistencies are observed between mice and rats.

The underlying causes of sex differences in MI and HF animal models have been examined at the transcriptome level. In a microarray study analyzing cardiac gene expression changes 3 days after MI, female mice displayed increased induction of genes involved in angiogenesis, immune response, and extracellular matrix remodeling compared with male mice. In addition, female animals had a decreased amount of pathological responses. Female ERα knockout (αERKO) mice experiencing β knockdown (ERKO) mice resulted in smaller infarcts. This was not observed in αERα knockout mice. Although this result may provide a general explanation for sex differences in cardiac remodeling observed after MI in men and women, the mechanism is undoubtedly multifactorial.

ER signaling seems to play a role in mediating post-MI responses. Female ERβ knockout (βERKO) mice experiencing chronic HF after MI exhibited increased mortality and altered expression of calcium-handling proteins, suggesting that ERβ plays a critical role during HF. In addition, estrogen treatment of ovariectomized ERα knockout (αERKO) mice resulted in smaller infarcts. This was not observed in βERKO animals, further implicating that ERβ is important for mediating estrogen’s effects in the heart post MI. These results are complicated by the observation that estrogen treatment increased post-MI mortality in WT controls and αERKO mice, suggesting that estrogen signaling is not universally protective. Although reports using ERKO mice suggest that these receptors have important and distinct cardiac roles, these studies are confounded by the systemic effects of global ER deletion as αERKO mice have increased levels of circulating estrogen, are obese, and are insulin resistant and βERKO.

Table 2. Sex Differences in Animal Models of Myocardial Infarction

<table>
<thead>
<tr>
<th>Model</th>
<th>Age</th>
<th>MI Stage/Ischemia Protocol</th>
<th>Reported in Male Animals</th>
<th>Reported in Female Animals</th>
<th>Molecules/Pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary artery ligation—C57BL/6J mice124</td>
<td>12 wk</td>
<td>Acute: 1, 2, 4, 7, or 14 d after MI; chronic: 12 wk after MI</td>
<td>Acute: Mortality was higher; chronic: displayed worse cardiac function and more dilation</td>
<td>Not addressed</td>
<td></td>
</tr>
<tr>
<td>Coronary artery ligation—C57BL/6J mice126</td>
<td>12–15 wk</td>
<td>No difference in infarct size</td>
<td>Exhibited better survival and were less likely to progress to dilation</td>
<td>Greater induction of genes involved in angiogenesis, ECM remodeling, and immune response observed in infarcted female hearts</td>
<td></td>
</tr>
<tr>
<td>Coronary artery ligation—Sprague Dawley rats133</td>
<td>12 wk</td>
<td>1 and 6 wk post MI</td>
<td>Greater increases in thickness of noninfarcted regions and restrictive diastolic filling patterns</td>
<td>No difference in infarct size</td>
<td>Not addressed</td>
</tr>
<tr>
<td>Coronary artery ligation—Sprague Dawley rats137</td>
<td>10–12 wk</td>
<td>4 wk post MI</td>
<td>No difference in mortality rates</td>
<td>Female rats exhibited more pronounced LV dilation than male rats</td>
<td>Not addressed</td>
</tr>
<tr>
<td>I/R injury—129J and C57BL/6 mice141,142</td>
<td>12–24 wk</td>
<td>Ex vivo: 30-min perfusion, 20-min ischemia followed by 40-min reperfusion</td>
<td>Injury was greater in Iso or calcium-treated hearts</td>
<td>No difference under basal conditions</td>
<td>NOS signaling</td>
</tr>
<tr>
<td>I/R injury—NCX Tg mice and non-Tg littermates146</td>
<td>32–36 wk</td>
<td>Ex vivo: 30-min perfusion, 20-min ischemia followed by 40-min reperfusion</td>
<td>Postischemic function was decreased in Tg male mice</td>
<td>No difference under basal conditions; Tg sex difference was abolished by OVX</td>
<td>Estrogen</td>
</tr>
<tr>
<td>I/R injury—Sprague Dawley rats148</td>
<td>11–15 wk</td>
<td>Ex vivo: 30-min occlusion followed by 150-min reperfusion</td>
<td>Infarct sizes were larger; gonadectomy of both sexes produced opposite results</td>
<td>Sex hormones, particularly androgens downregulating apoptosis in response to MI</td>
<td></td>
</tr>
<tr>
<td>I/R injury—Sprague Dawley rats150</td>
<td>10 wk</td>
<td>In vivo: 30 min of ischemia by clamping a coronary artery, 24 h reperfusion</td>
<td>Infarct area and percentage of apoptosis was greater</td>
<td>Differential regulation of apoptosis and autophagy pathways by an unknown mechanism</td>
<td></td>
</tr>
<tr>
<td>I/R injury—Sprague Dawley rats153</td>
<td>Not specified</td>
<td>Ex vivo: 15-min equilibration, 27-min ischemia followed by 40 min of reperfusion</td>
<td>Increased inflammatory response in response to injury</td>
<td>Postischemic cardiac function was significantly improved</td>
<td>Inflammatory signaling mediated by p-38 MAPK activation</td>
</tr>
<tr>
<td>Isolated cardiac myocytes—C57BL/6J mice155</td>
<td>8–10 wk</td>
<td>Cells treated with 100 μmol/L H2O2 for 30 min</td>
<td>Treated cells exhibited greater survival, decreased LDH release, apoptosis, and necrosis</td>
<td>Akt and caspase signaling</td>
<td></td>
</tr>
</tbody>
</table>

ECM indicates extracellular matrix; I/R, ischemia-reperfusion; Iso, isoproterenol; LDH, lactate dehydrogenase; LV, left ventricle; MAPK, mitogen-activated protein kinase; MI, myocardial infarction; OVX, ovariectomy; and Tg, transgenic.
mice exhibit hypoxia and hypertension. Other models, however, provide evidence of the cardioprotective effects of estrogen. For example, estrogen treatment of ovariectomized rats 24 hours after MI resulted in increased expression of connexin 43, which allowed for critical cell gap junctions to be maintained and reduced fatal ventricular arrhythmias. Ovariectomy alone in WT mice worsened LV function and dilation, suggesting a protective role for estrogen. In contrast, testosterone worsened cardiac function in both intact and ovariectomized WT female mice, and castration of WT male mice decreased the amount of cardiac ruptures and improved cardiac function.

Histone deacetylases (HDACs) are key modulators of MI by regulating the activity of cardiac transcription factors, such as myocyte enhancer factor 2. Although HDACs have become therapeutic targets for cardiac hypertrophy, they seem to have sex-specific effects, which should be considered. Female, but not male, HDAC 5 or 9 knockout mice were protected against pathological cardiac remodeling after MI in an Erα-dependent manner. Thus, active class II HDACs repress Erα expression and seem to promote pathological cardiac remodeling specifically in female mice. Increased Erα expression caused by HDAC inhibition could protect female mice by regulating expression of genes, such as vascular endothelial growth factor, thus promoting angiogenesis. These data provide a novel explanation for how cardiac Erα expression is regulated post MI and give insight into the potential protective effects of HDAC inhibitors in humans, particularly in women.

**Ischemia/Reperfusion Studies**

Animal models using ischemia/reperfusion (I/R) provide valuable mechanistic information in terms of surgical reconstitution of blood flow to damaged cardiac tissue after MI in humans. Under basal conditions, there was no difference in susceptibility to I/R injury or infarct size between male and female mice. Despite this lack of difference at baseline, overexpression of NCX increased I/R injury in male mice but not in female mice, suggesting that female mice are less prone to injury when calcium homeostasis is perturbed. Exposure to isoproterenol or calcium pretreatment supports this conclusion; I/R injury is increased to a greater extent in male mice compared with female mice. The hearts of male mice treated with isoproterenol before I/R accumulate more intracellular sodium than do female hearts. In contrast, several other studies report that under basal conditions, female hearts displayed preserved contractile function, smaller infarct size, and fewer apoptotic cells compared with male hearts. These differences are also apparent at the cellular level as intracellular sodium tended to be higher in isolated cardiac myocytes from male mice, and female cardiac myocytes survived at a higher rate than male cardiac myocytes when exposed to an oxidative stressor. The variation in results of I/R injury between sexes could be because of differences in the I/R experimental protocol as many of these studies used different lengths of time to induce an ischemic event.

Examination of the mechanisms mediating sexual dimorphisms in response to I/R also support a role for calcium homeostasis and involves differences in NO signaling, specifically S-nitrosylation between sexes. NO production was higher in the hearts of female mice at baseline, and female cardioprotection in I/R injury models after isoproterenol exposure was lost on treatment with the L-NAME. Furthermore, female hearts lacking NOS I or NOS III exhibited increased contractility and were also not protected from I/R injury. NO may be mediating this effect by altering intracellular sodium levels as L-NAME treatment of hypercontractile hearts blocked differences in intracellular sodium levels, which were higher in male hearts. Nonspecific NOS inhibition also increased calcium in female hypercontractile hearts to levels similar to male hearts, a process that is regulated by the S-nitrosylation state of the L-type calcium channel. Thus, sex differences in I/R injury animal models may be attributable to increased intracellular calcium in male hearts.

Survival and apoptotic signaling pathways also play important roles in mediating sex differences during ischemic events. For example, cardiac myocytes isolated from female mice displayed higher levels of phosphorylated Akt both before and after oxidative stress. Caspase 3 activity was also lower in female rat cardiac myocytes treated with an oxidative stressor compared with male counterparts, possibly contributing to the greater survival observed in the female cells. Apoptotic signaling differences are also observed in the whole heart. After I/R injury, levels of the antiapoptotic protein, B-cell lymphoma 2, were significantly lower, whereas levels of the proapoptotic protein, Bax (Bcl-2 associated X protein), were unchanged in male but not in female hearts. Furthermore, phospho-p38 levels that promote apoptosis were significantly increased in male hearts after I/R, whereas in the hearts of female mice, increased autophagy was observed.

In I/R injury studies, sex hormones promote opposing effects in terms of infarct size. Ovariectomy of female rats led to larger infarct sizes, but estrogen supplementation attenuated this response. Testosterone, however, aggravated the response to I/R by downregulating the antiapoptotic protein B-cell lymphoma 2-XL leading to the enhanced cardiac injury observed in male mice. BERKO mice (but not αERKO mice) displayed less functional recovery if exposed briefly to isoproterenol before I/R injury making them similar to what was observed in WT male mice, suggesting that cardioprotection in female mice is mediated by ERβ signaling. Furthermore, hearts of βERKO mice had altered expression of multiple metabolic genes compared with WT and αERKO female mice, which could explain functional differences in response to I/R injury. The protective effects of estrogen are also observed in a cellular model of ischemia as estrogen treatment led to decreases in intracellular calcium and sodium during metabolic inhibition in male cardiac myocytes, abolishing observed sex differences.

The complexity of MI or I/R injury in mouse models discussed here parallels that of humans by producing more severe phenotypes in male mice, but this is less apparent in rats. In addition, female mouse hearts display less cell death after injury and are less likely to progress to HF, which is in agreement with human studies. The role of estrogen in mediating the observed protection in female mice continues to be unclear because estrogen supplementation has conflicting effects on MI outcome, which is also representative of reports in humans.
Cardiac Hypertrophy

Pathological stimuli, such as hypertension, aortic stenosis, or cardiac injury, result in cardiac hypertrophy that may initially compensate for disrupted function; however, prolonged exposure to these pathological stressors leads to decreased cardiac function, increased fibrosis, and an increased risk of HF. The phenotypic appearance and development of cardiac hypertrophy are distinct between men and women (Table 3) and are modulated by hormones and rodent diets that contain high levels of phytoestrogenic compounds.

Pathological Hypertrophy Induced by Pressure Overload

In humans with aortic valve stenosis or hypertension, LV hypertrophy develops to maintain functional cardiac output and ≤50% of those patients will progress to HF. Pressure overload is commonly studied in animal models by banding either the ascending or transverse aorta and is particularly useful because development of cardiac hypertrophy is gradual and progresses to HF. As with other CVD animal models, sexual dimorphisms are apparent in pressure overload studies with male animals consistently developing more severe disease symptoms in a variety of experimental settings, consistent with studies in men and women (Figure 4). In studies of both mice and rats, male hearts developed eccentric cardiac hypertrophy, whereas female hearts exhibited concentric hypertrophy as observed in men and women with aortic stenosis. Male hearts exposed to pathological stimuli were more likely to exhibit decreased contractility and increased fibrosis compared with their female counterparts. Although female hearts hypertrophied after aortic constriction, sometimes even to a similar degree as the male hearts, cardiac function was preserved over time, and cardiac expression of fetal genes, such as atrial natriuretic peptide,

<table>
<thead>
<tr>
<th>Model</th>
<th>Age</th>
<th>Hypertrophic Stimulus</th>
<th>Reported in Male Animals</th>
<th>Reported in Female Animals</th>
<th>Molecules/Pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure overload—Wistar rats</td>
<td>3–4 wk</td>
<td>Aortic banding: 6 and 20 wk</td>
<td>By 20 wk, progressed to heart failure</td>
<td>Hypertrophic response is similar after 6 wk</td>
<td>Not addressed</td>
</tr>
<tr>
<td>Pressure overload—Wistar rats</td>
<td>Not specified</td>
<td>Aortic banding: 6 wk</td>
<td>Exhibited increased expression of fetal genes</td>
<td>Extent of hypertrophy was similar between sexes</td>
<td>Not addressed</td>
</tr>
<tr>
<td>Pressure overload—C57BL/6J WT and ERβ KO mice</td>
<td>8 wk</td>
<td>Aortic banding: 9 wk</td>
<td>WT male mice developed more hypertrophy, fibrosis, and heart failure; this difference was abolished on ERβ KO</td>
<td>Estrogen signaling through ERβ regulating apoptosis and fibrosis</td>
<td></td>
</tr>
<tr>
<td>Pressure overload—B6D2/F1 mice</td>
<td>5 wk</td>
<td>Aortic banding: 4 wk</td>
<td>Exhibited more fibrosis</td>
<td>Extent of hypertrophy was similar between sexes</td>
<td>CaMKII activation regulating MEF2 transcription</td>
</tr>
<tr>
<td>Volume overload—Sprague Dawley rats</td>
<td>8 wk</td>
<td>AV fistula: 8 wk</td>
<td>Increased mortality and development of heart failure</td>
<td>Not addressed</td>
<td></td>
</tr>
<tr>
<td>Volume overload—Sprague Dawley rats</td>
<td>6 wk</td>
<td>AV shunt: 4 or 16 wk</td>
<td>By 16 wk, exhibited decreased cardiac function, which progressed to heart failure</td>
<td>Maintained cardiac function by 16 wk</td>
<td>Estrogen upregulating phospho-Bcl2 to attenuate apoptosis and β-adrenergic signaling in female hearts</td>
</tr>
<tr>
<td>Chemical—C57BL/6J mice</td>
<td>4 mo</td>
<td>Iso treatment: 7 d</td>
<td>Developed less hypertrophy</td>
<td>Developed less hypertrophy</td>
<td>Estrogen broadly regulating the activation of kinase signaling</td>
</tr>
<tr>
<td>Chemical—Sprague Dawley rats</td>
<td>Weight matched (270–290 g)</td>
<td>Acute Iso treatment (1 μmol/L) of isolated myocytes</td>
<td>In response to Iso, cells displayed greater cell shortening, calcium current density, and cAMP production</td>
<td>β-adrenergic signaling differences between sexes</td>
<td></td>
</tr>
<tr>
<td>Genetic HCM mice</td>
<td>4 and 10 mo</td>
<td>R403Q mutation in α-MYH</td>
<td>By 10 mo, developed cardiac dilation and dysfunction</td>
<td>Developed hypertrophy by 4 wk, maintained function by 10 wk</td>
<td>Not addressed</td>
</tr>
<tr>
<td>Genetic HCM mice</td>
<td>12 wk</td>
<td>Truncated cTnT or missense R92Q mutation in cTnT</td>
<td>In both mouse models, treatment with Iso or PE resulted in sudden cardiac death in all male mice</td>
<td>At baseline, R92Q hearts were larger, exhibited decreased hypertrophic gene expression and fibrosis</td>
<td>Not addressed</td>
</tr>
<tr>
<td>Genetic HCM mice</td>
<td>13 wk</td>
<td>Knockin model with a heterozygous point mutation in MYBPC3</td>
<td>Isolated cardiac myocytes and myofilaments displayed reduced maximal force generating capacity</td>
<td>Not addressed</td>
<td></td>
</tr>
</tbody>
</table>

AV indicates arteriovenous; Bcl2, B-cell lymphoma 2; cAMP, cyclic AMP; CaMKII, calcium calmodulin–dependent kinase; cTnT, cardiac troponin T; ERβ, estrogen receptor-β; HCM, hypertrophic cardiomyopathy; KO, knockout; Iso, isoproterenol; MEF2, myocyte enhancer factor 2; MyHC, myosin heavy chain; PE, phenylephrine; and WT, wild-type.
male heart. However, ERβ gene expression was not detectable in adult cardiac myocytes from male mice or rats of either sex, suggesting that this ERβ signaling is mediated in cardiac fibroblasts, endothelial, or vascular cells.174 (E.K. Pugach and C.L. Blenck, unpublished data, 2016).

Evidence exists for other signaling pathways involved in mediating sex differences observed in response to pressure overload. The calcium calmodulin–dependent kinase-myocyte enhancer factor 2 pathway may be important as calcium calmodulin–dependent kinase-phosphatase compartmentalization differed between sexes after pressure overload, leading to differences in myocyte enhancer factor 2 activation, which can promote cardiac hypertrophy.168 In addition, cardiac expression of a dominant-negative form of p38α MAPK resulted in severe hypertrophic development and mortality in female mice but not in male mice after pressure overload.175 However, ovariectomy abolished the increased hypertrophic responses observed in transgenic female mice.175 In addition, male rats exhibited increases in NOS1 expression, a factor that has consistently been upregulated in HF, after aortic banding much earlier than female rats, once again demonstrating the importance of NO in mediating cardiac sexual dimorphisms.176 Complex signaling mechanisms mediate sexual dimorphisms associated with pressure overload hypertrophy, and further studies are required to elucidate interactions among those implicated.

**Pathological Hypertrophy Induced by Volume Overload**

Anatomic defects that cause conditions, such as mitral or aortic valve regurgitation, result in increased ventricular blood volume and cause the thickness of ventricular walls to increase to maintain cardiac function.177 Similar to pressure overload, the consequences of volume overload were less severe in female animals; male animals exhibited decreased contractile function and increased mortality rates and were more likely to progress to HF.178,179 Male animals also displayed higher levels of plasma catecholamines and increased cardiac expression of angiotensin II (Ang II) type 1 receptor and proapoptotic proteins, such as BAX and caspase 3 and 9.179,180 This sex-specific alteration in apoptotic signaling in animals agrees with increased fibrotic gene expression observed in cardiac biopsies from men but not from women experiencing LV hypertrophy caused by aortic stenosis.181

As in pressure overload, estrogen protects from development of pathological hypertrophy induced by volume overload. Ovariectomy of female rats resulted in more pronounced hypertrophy that progressed to ventricular dilation.182 Interestingly, supplementation of ovariectomized rats with 17β-estradiol did not fully attenuate the development of hypertrophy, suggesting that other ovarian hormones may be mediating protection.179 However, this contradicts studies that demonstrated rescue of cardiac function with estrogen administration in similar volume overload models.180,183 In addition, estrogen regulates apoptosis during this hypertrophic response as ovariectomized rats exhibited increased cardiac proapoptotic signaling similar to levels observed in male rats, but this response was abrogated with estrogen supplementation.180 Estrogen also regulates fibrosis in a sexually dimorphic manner in that collagen gene expression increased on estrogen
treatment in male but decreased in female adult rat cardiac fibroblasts, which is consistent with sex differences observed clinically.181

Estrogen may also be regulating other signaling pathways in response to volume overload. Male but not female rats displayed decreased cardiac expression of β-adrenergic receptors (β-ARs) and adenylyl cyclase in response to arterial shunt.182 However, ovariectomized female rats also exhibited decreases in β-AR and adenylyl cyclase expression in response to volume overload, and estrogen supplementation brought these values back to levels observed in intact female rats, suggesting that estrogen may maintain cardiac function in response to volume overload stress by upregulating the β-AR signaling pathway.182

**Chemical Induction of Cardiac Hypertrophy**

Chemical agonism of cardiac pathways, such as the β-adrenergic and Ang II pathways, also promotes pathological cardiac hypertrophy. After 1 week of isoproterenol treatment, male mice developed greater cardiac hypertrophy than female mice and also displayed altered contractile function.183 Sex differences are also apparent at the cellular level as isoproterenol treatment increased SR calcium levels in male but not in female cardiac myocytes.184 In addition, isoproterenol treatment elicited a greater increase in cyclic AMP production, cell shortening, and intracellular calcium transients in male cardiac myocytes.185,186 However, at higher isoproterenol concentrations, male but not female cardiac myocytes exhibited signs of calcium overload.186 Ang II promoted a different sex-specific response; cardiac myocytes isolated from aged female mice overexpressing Ang II were more prone to contractile dysfunction than their male counterparts.187 However, these female cells also exhibited stable SR calcium stores, whereas the male cells displayed increased spontaneous contractility, once again suggesting that female myocytes are protected from calcium overload by unknown mechanisms.187

Acute estrogen treatment of isolated male cardiac myocytes also treated with isoproterenol inhibited cyclic AMP production and increased peak calcium.188 Similarly, myocytes isolated from the hearts of ovarioctomized rats treated with isoproterenol exhibited increased calcium transients, force of contraction, and protein kinase A activity compared with sham controls, but estrogen supplementation restored these parameters to sham levels.189 Myocytes from ovarioctomized rats displayed altered expression of β-ARs compared with sham controls, which could explain functional differences in response to isoproterenol.190 Estrogen seems to protect from Ang II–induced hypertrophy as ovarioctomized mice supplemented with estrogen during Ang II treatment developed less hypertrophy and fibrosis than the vehicle-treated animals.191 This response seems to be mediated by ERβ signaling because ovarioctomized βERKO animals supplemented with estrogen were no longer protected from developing hypertrophy or fibrosis as was observed in αERKO or WT ovarioctomized animals.191

**Genetic Hypertrophic Cardiomyopathy Animal Models**

Hypertrophic cardiomyopathy (HCM) is a well-characterized autosomal dominant genetic disease, which affects ≈1 in 500 individuals. Disease-causing mutations have been found in at least 11 different genes that are important for maintaining contractile function, such as components of the sarcomere and calcium-handling genes. A variety of different mouse models have been created that harbor disease-causing mutations reported in humans.192 One of the most commonly used mouse models has a missense mutation (R403Q) in α-MyHC, which when present in the β-MyHC human gene produces a severe form of HCM.193 These mice develop HCM similar to human patients in a manner that is modulated by sex over time.193 Although male and female mice both developed cardiac hypertrophy to a similar extent at 4 months of age, only the male mice displayed LV dilation and systolic dysfunction at 10 months.193 In young HCM mice, the R403Q α-MyHC mutation enhanced LV myofilament performance, but myofilament function was not different between sexes.194 However, in a more recent study analyzing older mice with established HCM, 10-month-old female HCM mice had larger hearts, and their trabeculae were more sensitive to calcium than their male counterparts.51 This difference in calcium sensitivity could be attributed to higher expression of sarco/endoplasmic reticulum calcium transport ATPase in female HCM hearts or the reduced phosphorylation of cardiac troponin T (cTnT) observed in the male HCM hearts.51 Furthermore, the signaling pathways activated in this HCM model seem to be different between sexes as HCM male mice also expressing a constitutively activated glycogen synthase kinase 3β exhibited contractile dysfunction, decreased sarco/endoplasmic reticulum calcium transport ATPase expression, and premature death, but this was not observed in their female counterparts.195 Interestingly, removal of phytoestrogens from rodent chow abrogated the severe dilated cardiomyopathy observed in HCM male mice.160 However, female HCM mice did not progress to HF on either phytoestrogen-free or soy-based diets.160 The predominant phytoestrogen in soy, genistein, activates apoptotic pathways in the male HCM heart contributing to the development of cardiac dysfunction.73 In addition, estrogen treatment was not protective in either male or female HCM animals and actually increased mortality in phytoestrogen-fed male HCM animals.73

Mice harboring a truncated cTnT protein displayed smaller ventricles and contractile dysfunction, but not fibrosis, where transgenic mice with a missense mutation (R92Q) in the cTnT gene exhibited severe fibrosis and induction of hypertrophic markers.196 In addition, although no significant differences at baseline were observed in the truncated cTnT animals from either sex, the male R92Q animals displayed smaller heart weights, increased expression of hypertrophic markers, and increased fibrosis compared with their female counterparts.196 Both of these cTnT models displayed sex differences with respect to exposure to adrenergic stimuli. Treatment with either isoproterenol or phenylephrine resulted in sudden cardiac death of all male but not female HCM animals.196 Unlike the R403Q α-MHC model, estrogen seems to be cardioprotective in the R92Q cTnT female animals. Ovariectomy further decreased contractile function and myocardial energy metabolism, but estrogen supplementation restored these parameters and reduced cardiac oxidative stress.197 In another model
of HCM, in which mice carry a point mutation in myosin-binding protein C, isolated cardiomyocytes and myofilaments from male animals exhibited reduced maximal force-generating capacity compared with female animals.198

Although the mechanisms for the observed sex differences in genetic HCM models are not well understood, they should be taken into account when choosing a genetic model of the disease. Directly extrapolating results from genetic HCM animal studies to humans should also be done carefully because of the phenotypic diversity of HCM patients, which is not as apparent in the animal models.199 Whether this limitation of HCM animal models is due to unknown modifiers of the human disease or a result of the difference in MyHC isoform predominance between mice and humans, caution should be taken when relating results to the human population.

In addition, it is not clear how the observed sex differences in animal HCM models are related to humans because understanding the effect of sex on the development and mortality associated with HCM is complicated by women being diagnosed later in life because of multiple environmental factors, such as clinical screening biases.200

Conclusions

During the past 20 years, awareness of heart disease in women has dramatically increased, but gaps remain in knowledge among women about their cardiovascular risk. Increasing our knowledge of sex differences in basic cardiac physiology is critical for effectively treating patients of both sexes having CVD. Human and animal studies have demonstrated that cardiovascular sexual dimorphisms exist in normal and diseased states from the level of cardiac myocyte to the whole heart. Despite several differences in cardiac physiology compared with humans, small rodent models have been extremely useful for better understanding the mechanisms responsible for mediating these observed sex differences. At baseline, differences in excitation–contraction coupling and mitochondrial function are apparent between the sexes, providing evidence that mechanisms involved in maintaining cardiac function are in place before the onset of any disease. Estrogen is believed to mediate this protection both before and after disease onset, but the exact mechanism is still not well understood and seems to be context dependent because estrogen supplementation can also be detrimental in some cases. As observed in humans, female animals are generally protected from developing multiple CVDs in genetic, surgical, and chemically induced models. In many disease states, such as cardiac hypertrophy or hypertension, this protection in female animals or increased risk in male animals is lost on the depletion of endogenous sex hormones. To appropriately investigate the mechanisms underlying CVD development, biological sex is an important experimental variable that needs to be addressed both in basic and clinical research studies. Although this review cited many different studies that focused on cardiac sex differences, most studies have not taken sex into account. More research needs to include animals of both sexes not only to better understand the responsible signaling mechanisms but to also ensure that therapeutics will work effectively in both men and women. With the new guidelines recently released by the National Institutes of Health that require biological sex to be included as a potential experimental variable in vertebrate animal and human studies, more insight into mechanistic studies of the sexual dimorphisms observed in the cardiovascular system may be gained.

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Disclosures

None.

References


Sex Differences in Animal Models


Sex Differences in Animal Models


The Importance of Biological Sex and Estrogen in Rodent Models of Cardiovascular Health and Disease
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