The Effects of Biological Sex and Diet on the Development of Heart Failure

John P. Konhilas, PhD; Leslie A. Leinwand, PhD

Biological sex and gender have emerged as prominent players in cardiovascular health. It is widely appreciated that male and female hearts are different both at baseline and in response to numerous stimuli (for a recent review see Llamas et al\(^2\)). In general, male hearts respond less well to pressure or volume overload, to myocardial infarction (MI), and to aging. In considering the mechanisms that underlie sexually dimorphic cardiac traits, estrogen is an obvious candidate. However, data in the literature conflict on this point. The findings that cardiovascular risk increases when estrogen production ceases and that ovariectomy prevents the profound effects on CVD in a sex-specific manner.

Clinical Impact of Sex and Congestive Heart Failure

Although the vast majority of clinical and laboratory studies have been carried out in males, a growing body of literature directly addresses sex-specific differences in CVD and outcomes. Premenopausal women consistently have a better prognosis than men in response to hypertension, aortic stenosis, and hypertrophic cardiomyopathy. The hearts of women with these disorders maintain adequate or elevated cardiac function, whereas men typically demonstrate increased chamber dilation and wall thinning, both of which contribute to the observed poor contractility.\(^1\) The same is also true for the clinical population suffering from congestive heart failure (CHF); women have a better rate of survival than men even when adjusted for severity of cardiac function.\(^1\) Adjustment for the severity of cardiac function during CHF is an important distinction because women typically demonstrate preserved ejection fraction (\(\geq 50\%\)) compared with men.\(^1\) Nevertheless, after the age of 65 years, the death rate in the United States as a result of CVD in women is greater than that in men.\(^1\) Moreover, despite reports in the clinical literature stating that females have a greater immediate (first 30 days) death rate as a result of an ischemic event (a strong predictor of CHF), the long-term prognosis is better for women than for men.\(^1\)

However, another study shows that, despite an increasing incidence and prevalence of CHF with age for both men and women, the rate in men always exceeds that in women independent of age.\(^1\) In the same study, no differences between men and women were found in CHF prognosis.\(^1\) Another publication, which cited a self-reported heart failure survey administered by the National Center for Health Statistics in 1999, reports a greater prevalence of CHF in men than women 65 to 74 years of age, but after age 74 years this difference significantly diminishes.\(^1\)

These apparently inconsistent reports underscore the difficulty in interpreting the CHF death rate literature and any sexual dimorphisms that may be revealed by these studies. A potentially major source of error contributing to these discrepancies lies in the definition of the types of CHF being studied. CHF, for example, is a syndrome that occurs as an end result of many CVD causes, and thus a clear definition remains elusive.\(^1\) CHF categorization largely depends on stratification of symptoms and consequent interventional therapies despite serious attempts by investigators to adjust for these confounding factors.\(^1\) For example, the likelihood to seek medical attention and, if procured, the extent and intensity of the medical intervention could affect the outcome of these studies.\(^2\) Moreover, the general underrepresentation of women in large clinical trials prevents global interpretation of published results.

\(^*\)It is generally accepted that biological sex is defined as being chromosomally male or female, whereas gender is a function of biological sex, culture, behavior, and environment. For simplicity, we have decided to use the term sex in this review.

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_Circulation_ is available at http://circ.ahajournals.org

DOI: 10.1161/CIRCULATIONAHA.106.672006
Sex and Rodent Models of CHF

Apart from investigating the underlying cause of the apparent sex differences in CHF, investigators are challenged to find appropriate models to study the impact of sex on the development, response, and progression of heart disease. Many investigators have used rodents to model cardiac disease and the progressive deterioration to CHF. In general, these studies support the notion that male sex predisposes the animal to an earlier onset and worsened cardiac phenotype than that of females (for a summary, see the Table). When ventricular function is measured in rats 20 weeks after pressure overload induced by aortic constriction, males develop clinical signs of heart failure, with ventricular dilation, loss of concentric remodeling, elevated wall stress, and diastolic dysfunction, whereas females do not. The transition to CHF in males occurs despite the fact that females have elevated systolic pressures compared with males. A similar trend was observed in rats after MI or ischemia. In rats, the progression to CHF after MI is attenuated in females compared with males.6,27 Mice demonstrate a similar sex-dependent response to MI, such that male hearts rupture more readily and have significantly poorer left ventricular function, more prominent dilation, and significant myocyte hypertrophy compared with females.28

This is further validated in salt-sensitive Dahl rats that develop hypertension and significant hypertrophy when fed a high-salt diet. One week after MI, male and female rats were fed a high-salt diet and analyzed 4 weeks later. Although the degree of cardiac hypertrophy is similar in both females and males, females undergo concentric hypertrophy with no additional cavity dilation, whereas males experience eccentric hypertrophy and ventricular cavity dilation.29,30 Accordingly, these females demonstrate elevated contractile function compared with males. Moreover, using a high-salt diet to induce pressure overload illustrates how dietary intake can influence cardiac remodeling and the development of heart failure in a sex-dependent manner.

The spontaneously hypertensive heart failure (SHHF) rat is a well-studied model in which rats are spontaneously hypertensive and are either homo- or heterozygous for an obesity gene. Animals of both sexes develop heart failure independent of the obesity gene.6,41,42 Mice demonstrate a similar sex-compared with females.28

More prominent dilation, and significant myocyte hypertrophy, whereas males exhibit overt signs of CHF by 16 months of age versus 22 months in females.31,32 In lean SHHF rats, the progression to CHF is hastened in the males compared with the females, with males exhibiting overt signs of CHF by 16 months of age versus 22 months in females.31,32 In obese SHHF, the onset of CHF rats is much earlier, and, similar to their lean counterparts, obese SHHF males develop CHF before obese SHHF females.34

Sex Dimorphism in Murine Models of Familial Hypertrophic Cardiomyopathy

In the mouse, investigators have been able to model inherited genetic heart diseases such as familial hypertrophic cardiomyopathy (FHC), which has been shown to have lower penetrance in females.35 We and others have documented sex differences in transgenic mice expressing a mutant myosin heavy chain transgene corresponding to a human mutation in β-myosin heavy chain that causes FHC.36–38 Significant left and right ventricular hypertrophy are evident early in life, but the typical histological features associated with this disease and electrophysiological parameters are more pronounced in male hearts than in female hearts.36,39 In addition, the male mice develop progressive left ventricular dilation and impaired cardiac function, whereas female counterparts show increasing hypertrophy without dilation and maintain adequate ventricular function.37,38 The progression to a dilated cardiomyopathy seen in males is indicative of the transition from compensated to decompensated cardiac hypertrophy and heart failure, whereas the females remain in a compensated state.

Mice expressing mutations in the troponin T (TnT) gene (a central part of the contractile proteins in the heart) corresponding to mutations found in the human population also display sex-dependent characteristics.40,41 One of these lines expresses a TnT missense mutation in the heart and has smaller left ventricles (a characteristic found in the human population with this mutation42), but only in the males.40,43 The other line models a splice donor site mutation leading to a truncated TnT that lacks a tropomyosin-binding domain. Both sexes of this mouse model exhibit smaller left ventricles and severe diastolic and milder systolic dysfunction.40 However, agonist stimulation with angiotensin II (a potent stimulator of cardiac remodeling44) reveals a sex-dimorphic phenotype in that female mice expressing this truncated TnT develop hypertrophy, whereas their male counterparts do not.43 Interestingly, treatment with both phenylephrine and isoproterenol (adrenergic agonists) leads to sudden cardiac death in all male TnT transgenics but to only 1 death out of 10 females. These latter observations are intriguing because the impact of these mutations in the context of pathological

Table. Rodent Models That Display Sex-Specific Differences in CHF Development/Progression

<table>
<thead>
<tr>
<th>Animal</th>
<th>CHF Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats</td>
<td>Aortic constriction/pressure overload</td>
</tr>
<tr>
<td>Wistar</td>
<td>MI</td>
</tr>
<tr>
<td>Wistar/Sprague Dawley</td>
<td>MI/pressure overload (high-salt feeding)</td>
</tr>
<tr>
<td>Salt-sensitive Dahl</td>
<td>MI/pressure overload (high-salt feeding)</td>
</tr>
<tr>
<td>SHHF/Mcc-cp</td>
<td>Pressure overload (genetic)</td>
</tr>
<tr>
<td>Mouse</td>
<td>FHC transgene</td>
</tr>
<tr>
<td>HCM</td>
<td>Overexpression</td>
</tr>
<tr>
<td>TnT (R92Q/trunc-TnT)</td>
<td>Overexpression</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Overexpression</td>
</tr>
<tr>
<td>LPH/α</td>
<td>Superinhibitory (point mutation)</td>
</tr>
<tr>
<td>LPH/PPAR-α</td>
<td>(striated muscle)/PPAR-α knockout (global)</td>
</tr>
<tr>
<td>Relaxin</td>
<td>Knockout (global)</td>
</tr>
</tbody>
</table>

The table lists rat and mouse models of cardiovascular disease demonstrating premature development of CHF or death in males when compared to female counterparts. The left column lists the animal along with the specific type of rat or mouse model. The right column indicates the type of surgical or genetic intervention used to generate the model.
stimuli clearly depends on the sex of the animal, indicating an underlying sex dimorphism in adrenergic responsiveness.

**Estrogen and Congestive Heart Failure**

The loss of estrogen in women after menopause is strongly associated with an increase in morbidity and death from CVD and CHF.\(^4,14,15,45\) It follows that the majority of studies on sex dimorphisms in the cardiovascular system examine the ability of sex hormones, most often estrogen, to impart protection against CVD. However, improving the prognosis for CVD in women is not as simple as replacing the lost estrogen. In the Heart and Estrogen-Progesterin Replacement Study (HERS), estrogen plus progesterin had no effect on the death rate in women with CHF.\(^46\) Nevertheless, women in the β-Blocker Evaluation of Survival Trial (BEST) who took hormone replacement therapy (estrogen, progestin, or both) show benefits in the rate of death from CHF.\(^47\) The role of estrogen as a cardioprotective agent has been challenged by the findings of the Women’s Health Initiative (WHI) study as mentioned previously\(^7\) and by a more recent study by Vickers et al.\(^8\) Still, not all agree with the interpretations of the WHI because of the length of time after menopause that hormone replacement therapy was initiated. In a secondary analysis, women who initiated hormone replacement therapy within 10 years of menopause tended to show a reduced risk for CVD, a trend not seen in women >10 years after the onset of menopause.\(^48,49\) Moreover, there are studies in rodents that demonstrate a clear beneficial effect of estrogen replacement in gonadectomized rats and mice subjected to different cardiac pathological stimuli.\(^3,4,50–52\) Taken together, these data highlight the necessity of understanding the cellular and molecular actions of estrogen and how this relates to the sex dimorphisms in cardiovascular disease.

**Figure 1.** Schematic representation of estrogen action in the cardiomyocyte. Estrogen has been shown to impact cellular signaling through a number of pathways. The traditional genomic action of estrogen is believed to act through nuclear estrogen receptors (ERs) that transactivate genes. Estrogen has also been shown to act rapidly through non-genomic mechanisms by acting directly on growth factor receptors or recruitment of ERs by growth factor receptors. In addition, estrogen can bind and activate plasma membrane ERs and/or estrogen binding proteins, thereby inducing intracellular signaling cascades. Gene activation through ERs can occur by 3 mechanisms: (1) Estrogen can form a homo- or heterodimer complex with ERs to transactivate genes that contain estrogen response elements (ERE), (2) estrogen-ER complex can bind EREs through tethering by cofactors associated with activator protein 1/SP-1 sites, and (3) ERs can be recruited to EREs by the activator protein 1 complex in the absence of estrogen. eNOS indicates endothelial nitric oxide synthase; ERK1/2, extracellular signal-regulated kinase 1/2; JNK, c-Jun-N-terminal kinase; NO, nitric oxide; p38, p38 mitogen-activated protein kinase; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; SP-1, specificity protein 1; and TRE, TPA-responsive elements.

**Estrogen Receptors and the Heart**

Although the present review is not intended to be a comprehensive evaluation of the molecular and cellular mechanisms of estrogen, some details about estrogen action need to be highlighted. The classic or genomic view of estrogen action describes estrogen interacting with nuclear ERα and ERβ. This homo- or heterodimeric complex is then able to transactivate genes containing estrogen response elements (ERE). ERs are widely expressed and demonstrate distinct tissue expression patterns.\(^53\) The presence of functional ERα and ERβ in the heart suggests direct effects of estrogen on the myocardium.\(^54\) 17β-Estradiol, the major circulating estrogen, binds equally to both ERα and ERβ and exerts its action as a ligand-activated transcription factor. The nuclear estrogen-ER complex binds to EREs directly or indirectly through tethering with activator protein 1 or specificity protein 1 transcription factor sites.\(^55,56\) Once bound, the ER complex can recruit transcriptional cofactors including components of the transcriptional machinery (Figure 1).

Levels of ERα (mRNA and protein) are equivalent in hearts of both men and women. Levels of ERβ mRNA, on the other hand, are higher in male than in female hearts.\(^57\) The relevance of ER receptors to cardiac disease is implicated by the findings that both ERα and ERβ are upregulated during human aortic stenosis.\(^58\) Similarly, myocardium from patients with end-stage heart failure demonstrates elevated expression of ERα mRNA.\(^57\)

The functional differences between the receptors are being elucidated with the help of mice that lack either ERα or ERβ. Both male and female mice that lack ERα are completely sterile because of immature uterine development and improper pituitary function.\(^59\) In males, progressive deterioration of testicular tissue contributes to loss of sperm and ultimately sterility.\(^60\) The lack of ERβ has no effect on
fertility in males, whereas females exhibit suboptimal pregnancies.60

Cardioprotection in male and female hearts against ischemia/reperfusion injury requires the presence of both receptors.51,62 It appears, however, that ERβ mediates the sex difference in response to pressure overload63 and attenuates the transition to heart failure.64 Female ERβ-null mice show a more rapid development of CHF and increased mortality rate after MI.64 Although similar studies in mice that lack aromatase (the enzyme that converts testosterone to estradiol) have not been performed, increasing evidence indicates that treatment with an aromatase inhibitor leads to significant cardiovascular risk.65

Estrogen can also initiate cellular changes through nongenomic mechanisms. In many instances, nongenomic regulation occurs via ERs located in or adjacent to the plasma membrane or through other non-ER, plasma membrane–associated, estrogen-binding proteins.4,55 Through binding to membrane-bound ERs and estrogen-binding proteins, 17β-estradiol can induce intracellular signaling cascades such as activation of protein kinase C, extracellular signal–regulated kinase, and other members of the mitogen-activated protein kinase family to trigger biological functions.53 Similarly, estrogen signaling can mediate signaling by growth factors such as insulin-like growth factor 1, epidermal growth factor, or transforming growth factor-α.66 This may occur by direct recruitment of ERα by growth factor receptors either at the cellular membrane or through downstream signaling intermediates (Figure 1).59

Gonadectomy and Its Effect on the Heart

Early studies showed that the removal of sex hormones by gonadectomy in rats significantly depresses cardiac function and induces a shift in myosin heavy chain content to the slower isoform (V/β), indicative of a pathological shift.67 This shift can be reversed by sex-appropriate supplementation.58 In SHHF rats, ovariecotomy followed by estradiol treatment prevents left ventricular hypertrophy and hypertension, which delays the typical onset of CHF in these animals.52 Moreover, physiological replacement of estrogen in ovariectomized mice reduces infarct size after MI,69 and another study demonstrated that estrogen prevents deterioration in cardiac function in castrated male mice after MI.50 In these studies, the protective effects of estrogen are attributed to its vasodilatory effects. Indeed, it has been shown that estrogens cause vasodilation through both rapid increases in the vasodilating agent, nitric oxide, and the induction of nitric oxide synthase genes.70 Many of these effects of estrogens on vascular function are elicited through similar genomic and nongenomic mechanisms and will not be discussed further in the present review.71

However, cardioprotection by estrogen may be mediated through additional actions of estrogen. For example, an important nongenomic consequence of 17β-estradiol/ER receptor activation is tyrosine phosphorylation and subsequent stimulation of the insulin-like growth factor 1 signaling axis.72,73 This effect is mediated through ERα in many tissues, indicating further selective capacity of ER-mediated regulation.72 Initiation of the insulin-like growth factor 1 signaling cascade leads to activation of phosphatidylinositol 3-kinase (PI3-K) and downstream phosphorylation of Akt (protein kinase B). Akt is implicated as a central player in glucose metabolism, gene transcription, protein synthesis, and cell survival.74 As a mechanism of cardioprotection, one study shows that the levels of nuclear-localized phosphorylated-Akt (p-Akt) is greatest in young women compared with men or postmenopausal women.75 Furthermore, these investigators demonstrated the ability of 17β-estradiol to enhance nuclear localization of p-Akt in cultured cardiac myocytes. These cardioprotective effects of 17β-estradiol may be mediated, in part, by a PI3-K/Akt–dependent reduction in apoptosis.76 Estrogen may also impart cardioprotection by acting directly on large-conductance Ca2+-activated K+ channels in cardiac mitochondria, thus increasing activity and providing protection against ischemic events.77

Other Potential Mediators of Estrogen-Mediated Sex Differences

The ability of estrogen to exert its protective actions through activation of cell survival pathways does not preclude estrogen-mediated regulation of alternative beneficial pathways. Premenopausal women typically show enhanced circulating lipid dynamics characterized by more rapid production of VLDL particles that also differ in their molecular characteristics compared with men.78,79 Because the hydrolysis of lipoproteins including VLDL particles is largely mediated by lipoprotein lipase, the elevated lipoprotein lipase activity in female muscle and adipose tissue can further explain these differences in VLDL dynamics.80–82 These differences in VLDL dynamics may partially explain the differential impact of plasma lipoprotein profiles on cardiovascular risk in males and females. In addition, the more efficient hydrolysis of VLDL particles coupled with a more efficient substrate (triglyceride-rich, VLDL particles) in females may provide a protective mechanism during times of increased energy demand such as occurs during CHF.

In addition to lipoprotein particle dynamics, other factors are emerging as important players in the sex dimorphisms of CHF. Estrogen directly or indirectly activates AMP-activated protein kinase (AMPK) by phosphorylation in many tissues, including adipose tissue and cardiac muscle.83,84 AMPK increases glucose transport and glycolysis in addition to promoting free fatty acid (FFA) uptake and metabolism. Given the contribution of AMPK to glucose uptake and oxidative metabolism during cardiac stress (for review, see Arad et al86) and the ability of estrogen to modify AMPK activity,83 it can be hypothesized that females are more capable of mobilizing and activating AMPK during cardiac stress as a result of elevated levels of circulating estrogens than their male counterparts (Figure 2). Further examinations of the sex dimorphisms in AMPK regulation will answer 2 important questions: (1) Are females better able to increase FFA and VLDL-triglyceride flux and prevent the glycolytic shift during CHF by maintaining efficient FFA utilization? (2) Are females predisposed toward this glycolytic shift and able to avoid similar detriments from this glycolytic shift?
Figure 2. AMP-activated protein kinase regulation in the heart. AMP Kinase is a central regulator in fuel supply and energy generation. It responds to a variety of cellular stresses that impact the ratio of AMP to ATP in the cell. The ability to increase energy in response to cardiac stress makes AMPK an ideal candidate for imparting sex-specific responses to CVD. It is currently unknown how estrogen may play a role in regulation of AMPK. GLUT indicates insulin-regulated glucose transporter; PKF, phosphofructokinase; ACC, Acetyl-CoA carboxylase; CPT1, carnitine palmitoyltransferase 1; and MCD, malonyl-CoA decarboxylase.

Gene Expression Array

In the case of expression array analysis, a small number of nonfailing and failing human hearts were profiled, and the present study shows that sex is associated with substantial differences in gene expression. In the study by Boheler et al., 11 genes in failing hearts vary by sex, including calponin 1, insulin growth factor–binding protein 4, mitogen-activated protein 4 kinase 5, and cyclin-dependent kinase inhibitor. The directional change in gene expression depends on sex. For example, calponin 1 increases in females only with CHF, whereas Grp58 expression increases in females and decreases in males with CHF. In another study, expression array analysis of male and female mice subjected to pressure overload identified sex-dimorphic clusters of genes. Immunity/inflammation/stress response genes are largely represented in these up- and downregulated gene clusters. Specific genes in this class include chemokine receptor 10 and crypdtin-6, which are upregulated in males but downregulated in females, and APG-1 and cathepsin L, which are upregulated in females only. Interestingly, acute pressure overload results in more differentially expressed genes in males than in females, whereas chronic pressure overload results in a similar number of differentially expressed genes.

Transgenic Models Targeting Signaling Pathways

An approach to defining molecular players in CHF is the selective disruption or augmentation of signaling pathways in mice and assessment of cardiac phenotypes in both males and females. Such studies have uncovered some candidate mediators of the sex-specific differences in cardiac phenotypes. Although some of the genetic manipulations induce subtle differences between the sexes, those that result in a more severe phenotype, such as death, may better represent a first approach to understanding the cellular mechanisms behind these observations. In one such mouse model, investigators overexpressed tumor necrosis factor-α (TNF-α), a proinflammatory cytokine with pleiotropic biological effects, in the heart. By 6 weeks of age, male hearts demonstrate reduced cardiac function associated with left ventricular wall thinning and chamber dilation, whereas female hearts show left ventricular wall thickening but no change in ventricular chamber dimensions. Male death rate reaches ~50% by 20 weeks of age, compared with 4% in females.

The potential mechanism of this sex difference may be related to elevated circulating levels of TNF-α in postmenopausal women. In a recent study, estrogen replacement in ovariectomized rats decreases circulating TNF-α after ischemia/reperfusion injury, with a concomitant decrease in TNF receptor 2 and increase in TNF receptor 1. The combined effect of TNF receptor 2 downregulation with a concomitant TNF receptor 1 upregulation may be antiapoptotic in the heart after ischemia/reperfusion injury. The impact of estrogen on TNF-α in the heart may be partially mediated by its actions on cytokine production from other cells such as macrophages.

Another mouse was generated that overexpresses (by 4-fold) phospholamban, the inhibitor of sarcoplasmic reticular Ca²⁺ sequestration in the cardiac myocyte. In these animals, despite similar elevations of circulating catecholamines in both males and females, males develop significant cardiac hypertrophy and chamber dilation along with increased death rate by 15 months of age. At the same time point, females show no evident cardiac hypertrophy or chamber dilation. Only at 22 months of age do females exhibit clinical signs of CHF. Interestingly, despite significant differences in death rate at 15 months of age, both males and females demonstrate significant impairments in cardiac function (left ventricular fractional shortening and the velocity of circumferential fiber shortening) as measured by echocardiography. A mouse model that expresses a superinhibitory phospholamban results in a significant death rate between 2 and 16 weeks in males only. Similarly, pressure overload in mice that lack both α1AC- and α1B-adrenergic receptors results in an increased death rate in males only. Taken together, these data indicate a fundamental difference in the ability to contend with increased adrenergic drive between males and females despite a lack of evidence showing sex-dependent differences in adrenergic receptors.

In support of this sex effect, there are documented sex differences in Ca²⁺ handling proteins related to adrenergic stimulation. Although ovariectomized rats do not show a decrease in phospholamban mRNA levels, estrogen replacement elicits a significant increase in expression compared with ovariectomized and sham-operated animals. Similarly, the increase in Ca²⁺ fluxes across the ryandine receptor and Na⁺–Ca²⁺ exchange in ovariectomized rats is reversed by estrogen treatment. This latter effect of estrogen on Ca²⁺ flux may be the result of the impact of estrogen on protein kinase A activity.

In highly aerobic tissues with high fatty acid flux like the heart, cellular lipid balance is critical to normal function. Studies in mice that lack peroxisome proliferator-activator
receptor-α (PPAR-α), a nuclear receptor that targets fatty acid oxidation genes, have revealed significant sex differences. Pharmacological inhibition of carnitine palmitoyltransferase 1, a critical regulator in mitochondrial fatty acid import, proves lethal in male mice null for PPAR-α, whereas only 25% of female PPAR-α-null mice die. Interestingly, the hearts of male PPAR-α-null mice demonstrate marked lipid accumulation with severe systemic hypoglycemia, a characteristic found in those female mice that also died. Estradiol treatment in male PPAR-α-null mice reduces the rate of mortality in response to carnitine palmitoyltransferase 1 inhibition.

Skeletal and cardiac muscle overexpression of lipoprotein lipase in PPAR-α-null mice results in a similarly profound sex difference. In these mice, >50% of males die within 4 months of age, whereas the remaining males do not survive past 11 months of age. Females, on the other hand, survive for >12 months. Interestingly, the differences in plasma FFA levels as a result of the genetic manipulations cannot account for the extreme disparity in death rate. In both sexes, circulating FFAs are elevated and triglycerides are reduced. In these animals, there is an increase in skeletal muscle FFA content with a concomitant decrease in cardiac FFA content and no excessive lipid storage.

One implication from these studies is that female hearts may be better suited to utilize circulating FFAs or excess cellular lipids as a substrate for myocardial energy utilization. Alternatively, female hearts may be protected, possibly through enhanced clearance of accumulating cellular lipids, against FFA elevation compared with their male counterparts. The ability of estradiol to partially rescue male PPAR-α-null hearts from lipid accumulation and subsequent death after carnitine palmitoyltransferase 1 inhibition supports the role of estrogen as cardioprotective. Although the mechanism by which estradiol exerts this protection against metabolic stress is not known, studies have shown that PPAR heterodimerization with the retinoid X receptor is capable of activating estrogen-responsive genes in the absence of estrogen. This suggests that estrogen and PPAR-α may share some overlapping regulation. However, in the context of obesity, estrogen appears to inhibit the actions of PPAR-α by an unknown mechanism. Considering that PPAR-α plays a central role in determining circulating FFA and lipid characteristics, these studies provide a direct link between cellular lipid balance, cardiac disease, and sex. In addition, estrogen is strongly implicated as a central mediator of this sex difference.

Sex differences have also been documented in cardiac disease pathogenesis from transgenic models despite those genes not being previously implicated in human cardiac disease. For example, mice that lack one of the relaxin genes have been assessed for cardiac abnormalities. Relaxin, an insulin-like hormone, was originally identified as a critical factor during tissue remodeling associated with female reproduction but has also been found to impact cardiac contractility via cardiac atrial receptors. Although cardiac contractility is similar between male and female relaxin-null mice, males demonstrate a deficiency in diastolic filling, a precursor to severe cardiac disease.

The above studies show the potential utility of transgenic models to understand sex-specific differences in CHF development, but they also demonstrate the difficulty at hand for investigators. Each of the above models targets a distinct intra- or intercellular signaling pathway that leads to a distinct phenotype between the sexes. Because these signaling pathways can each be independently targeted by one or more factors, determining which factor leads to the sex dimorphism can become confounded by the potential interaction with other factors in vivo. Moreover, another level of complexity is introduced with models that have a global impact on circulating substrates (such as lipoprotein lipase and PPAR-α) and, therefore, necessitates the integration of other organ systems.

Cardiac Disease in Which Males Do Not Fare Worse Than Females

Transgenic models that lead to CHF and do not display sex differences may provide additional insight for investigators. For example, both sexes of mice overexpressing the cytoplasmic isoform of Ca2+/calmodulin-dependent protein kinase IIα display severe cardiac hypertrophy and dysfunction leading to ventricular dilation and premature death by 4 months of age (Elizabeth Luczak, University of Colorado at Boulder, personal communication, 2006).

There are also instances in which males do better in the face of CVD than females. For example, the impact of alcohol intake on death rate in patients with dilated cardiomyopathy is greater in females than in males. As a tool to identify mechanisms behind this difference, transgenic models exist in which females develop worse cardiac phenotypes than males. For example, both sexes of mice with cardiac-restricted overexpression of platelet-derived growth factor C develop hypertrophy, but only female animals showed dilated cardiomyopathy, heart failure, and sudden death. Similarly, only cardiomyocytes from females with cardiac overexpression of alcohol dehydrogenase respond to ethanol with reduced contractility. It should also be noted that the majority of studies using animal models of human CHF or models that overexpress or lack potential mediators of CHF have been carried out only in males or do not indicate which sex was studied.

Environmental Estrogens, Diet, and CHF

An important point to note is that all the studies described above do not take into consideration the impact of environmental factors such as diet on cardiovascular phenotypes. For example, most studies in laboratory rodents do not mention the specific diet fed to the animals, leading to the assumption that it is standard rodent chow. Of importance, standard rodent chow is high in phytosterogens, a potent biological compound that can affect cardiac phenotypes and will be discussed below. Understanding cardiovascular disease in the context of dietary intake, such as intake of nutrients that can mimic endogenous hormones like estrogen, may provide a unique perspective to the problem of sex differences in CVD risk, prognosis, and outcome.
Phytoestrogens
Recent attention is being given to environmental agents that can mimic endogenous hormones. Some of the more prominent environmental agents that fall into this category are plant estrogens (phytoestrogens), or isoflavones, which are typically ingested in the form of soy products. Perhaps more important for the scientific community is the fact that most rodent studies are performed on animals strictly fed a soy-based chow. In humans, the consumption of soy food is moderate and not a cause for concern, but the ingestion of soy dietary supplements is increasing, and they can contain extremely high levels of phytoestrogens. Interestingly, Asian men who eat a soy-rich diet and males and females who drink soy milk 3 times daily have circulating levels that are equivalent to those of rodents eating a soy diet (see Naaz et al112). Moreover, ≈25% of infants in the United States are fed soy formula. This is the highest percentage in countries in the Western world (for a review, see Chen and Rogan113). Serum concentrations of isoflavones in infants fed soy formula can be extremely high, reaching 200 times the concentrations in infants fed cow or breast milk and 10-fold higher than adults who eat a diet high in soy.113

Increased phytoestrogen intake in humans is partially fueled by assertions that consumption of soy products has a beneficial impact on blood lipid profiles.114,115 One meta-analysis demonstrates lipid-lowering effects of soy isoflavones independent of soy protein levels, implicating soy isoflavones as the active lipid-lowering ingredient.116 Other purported benefits of soy isoflavones relate to their estrogenic activity and include the lessening of menopausal symptoms, osteoporosis, and breast cancer.117 However, their health benefits remain under debate. Recently, the American Heart Association reversed its endorsement of soy products by stating it finds no effect of soy proteins or plant estrogens on lipids, blood pressure, or menopausal changes.117 Some of the detrimental effects ascribed to phytoestrogens include infertility in humans and animals of both sexes.118–121 Other reproductive effects that have been reported include male pseudohermaphroditism resulting from elevated estrogenic activity in the serum (from maternal intake of endocrine disrupters)122 and decreased sperm production in male rats on a high-phytoestrogen diet.123

It is clear that the biological impact of phytoestrogens is very broad and complex. Therefore, we need to examine more closely the molecular characteristics of phytoestrogen action. The dietary phytoestrogens in soy fall into the class of simple isoflavones, which are derived from flavanones and are structurally similar to estradiol.124 Both daidzein and genistein, and their metabolite, equol, bind to both ERα and ERβ.125 They can act as partial estrogen agonists or antagonists and also as nonhormonal compounds, much like their endogenous estrogen counterparts. As opposed to 17β-estradiol, phytoestrogen binds more strongly to ERβ than to ERα,126,127 but a number of experiments have shown that phytoestrogens can act through both receptors.127,128

However, some of their effects, like the negative impact of phytoestrogens on thymus size, are only partially blocked by an ER antagonist, which suggests that the actions of phytoestrogens and estrogen are not entirely overlapping.129 In other studies, estrogenic activity of the isoflavones depends on the estrogen environment.130 Some reports in the literature show that the 2 major soy phytoestrogens, genistein and daidzein, have differing effects on the actions of tamoxifen, an ER antagonist. For example, genistein can interfere with tamoxifen’s ability to inhibit tumor growth, whereas daidzein can enhance it.131,132 Dietary elements such as carbohydrate and fiber content can also influence isoflavone/phytoestrogen metabolism and thereby affect the bioavailability of these bioactive compounds.133,134

Genistein has been shown to improve endothelial function and upregulate antioxidant genes through ERK1/2 and NFκB signaling pathways, both properties that may be beneficial for cardiovascular health.135,136 In a recent study, genistein mimicked the actions of 17β-estradiol in its ability to localize phospho-Akt to the nucleus in cultured cardiac myocytes, a potentially cardioprotective outcome.75 It is unclear whether the mechanism of this cardioprotection by genistein is mediated through ERs or through some other mechanism. It is known that only a part of its biological activities is ER mediated.120 In fact, studies have found that genistein inhibits the growth of various cancer cell lines as a result of inhibition of protein tyrosine kinase activity.137–139 The mechanism of these antiproliferative effects appears to be mediated through genistein’s ability to inhibit NFκB activation by the Akt or Notch-1 signaling axis.139,140 However, genistein has also been shown to induce apoptosis through additional factors such as caspase-3 activation (an end-effector of apoptosis) and reduction in mitochondrial membrane potential.141–143

These latter actions of genistein would be detrimental to cardiac health. Given these conflicting properties of soy isoflavones, it is difficult to predict which bioactive activity of isoflavones will predominate in the context of CVD in both humans and laboratory rodents. We used an experimental model of hypertrophic cardiomyopathy (HCM) in which males progress to CHF to address whether the soy diet can influence cardiovascular health.37,38 In the present study, we report that male mice with HCM that were fed the traditional soy-based diet deteriorate to severe, dilated cardiomyopathy and have a number of pathological indicators, including fibrosis, induction of β-myosin heavy chain, inactivation of glycogen synthase kinase-3β, and activation of caspase-3.37,38 However, simply changing the diet to a milk-based (nonsoy) diet prevents these phenotypes.38 Female HCM mice do not exhibit clinical signs of severe cardiac disease on the soy diet, and they are not significantly affected by the dietary change.38 Next, we tested the hypothesis that phytoestrogens mediate these detrimental effects of soy on HCM by supplementing the casein diet with genistein and daidzein, the most abundant phytoestrogens in soy and the most common components of human soy dietary supplements.144 Feeding male HCM mice the phytoestrogen-supplemented diet is sufficient to recapitulate the worsened cardiac pathology as seen in male HCM mice eating the soy diet (unpublished observations). Among the pathological traits in phytoestrogen-fed HCM males is elevated apoptosis in the cardiac myocytes that mimics the known in vitro effects of genistein. Although we have not yet identified the general mechanism by which phytoestrogens induce this pathological cardiac phenotype, phytoestrogens
must act synergistically to potentiate aberrant signaling processes that are already activated in HCM mice. Therefore, the presence of phytoestrogens in the diet hastens the transition from a compensatory hypertrophic state to a deteriorating, decompensatory condition in male HCM mice.

Estrogen, Phytoestrogens and Lipid Metabolism

The thrust of preventative CVD intervention is based on studies that have consistently shown that an elevated LDL cholesterol level is an independent risk factor for CVD. However, lipid-lowering therapy and the reduction of cardiovascular risk are not equal among the sexes. Many factors contribute to the discrepancies in effectiveness of lipid reduction therapies between men and women, and these factors range from socioeconomic to biological. A recent assessment of previous randomized trials indicates that, although statin therapy reduces cardiovascular events equally in both men and women, women do not have the same reductions in death and stroke as their male counterparts. Moreover, in middle-aged and elderly subjects, men respond to restriction in total fat, saturated fat, and cholesterol differently than women. To further complicate matters, plasma triglycerides are better predictors of cardiovascular risk in women, whereas the LDL cholesterol concentration is a stronger predictor in men. However, this discrepancy disappears in older, postmenopausal women in whom LDL levels exceed those in men and become better correlated with cardiovascular risk.

Estrogen has been implicated as a mediator of differences in lipid profiles on the basis of studies showing that postmenopausal women have increased adiposity and susceptibility to metabolic disorders. In ovariectomized rodents, the accompanied obesity is reversed with estrogen administration by means of a reduction in adipocyte size and number. Adipose tissue expresses both ERα and ERβ receptors and can respond to estrogen by regulating lipogenesis, lipolysis, and adipogenesis.

Similarly, estrogen inhibits lipogenesis in cultured rat adipocytes and 3T3-L1 preadipocyte cell lines. Dietary genistein has antilipogenic effects in mice, which may be partially mediated through a negative effect on lipoprotein lipase. It was subsequently found that only male mice given nutritional doses of genistein (ie, doses corresponding to typical daily intakes of genistein in soy-based chow) demonstrate enhanced adipose deposition. The antilipogenic effects of estrogen are achieved at doses much higher than those attainable by diet. In the present study, the increase in adipogenesis is positively correlated with PPARα and PPARγ. Each of these factors is a critical player in oxidative metabolism but can have distinct results that depend on the context of genistein stimulation. The latter model of genistein action demonstrates that genistein can operate through the regulation of pathways that differ from those of estrogen.

Resveratrol

Resveratrol is a bioflavonoid that occurs naturally in grapes, peanuts, and blueberries and has recently gained attention with regard to its ability to extend lifespan in both invertebrate and vertebrate animals. It has also been suggested as a cardioprotective agent on the basis of data derived from large epidemiological studies (see Bradamante et al). However, resveratrol was originally characterized as a phytoestrogen on the basis of its ability to bind ERs. Because studies indicate that an average consumer is capable of absorbing physiological quantities of resveratrol from normal red wine intake, molecular characterization is necessary. Resveratrol exists as cis- and trans-isomers; the trans-isomer mediates most of its biological actions. It binds to equally to ERα and ERβ but with ~7000-fold lower affinity than 17β-estradiol. Resveratrol demonstrates genomic activity as indicated by activation of an ERE-luciferase reporter in transiently transfected Chinese hamster ovary K1 (CHO-K1) cells but shows higher activation with ERβ than ERα consistent with other phytoestrogen compounds like genistein. Nongenomic activity of resveratrol has also been described. In a recent study, resveratrol inhibited androgen- and ERα-dependent PI3-K activities in prostate cancer cell lines.

These nongenomic activities of resveratrol are of particular interest given the fact that resveratrol inhibition of the PI3-K pathway induces apoptosis and is thought to provide protective effects to certain cancers. Other chemoprotective effects by resveratrol are mediated through activation of p53 via ERK and p38 kinase, inhibition of inflammatory mediators, and downregulation of Bcl-2 and NFkB. These properties of resveratrol directly oppose the purported cardioprotective benefits. For example, activation of the PI3-K signaling cascade and protection against apoptosis by 17β-estradiol proves protective in cardiac myocytes. In addition, downregulation of Bcl-2 and increased apoptosis is a potential mechanism for the progressive deterioration in HCM.

Although no human studies that examine the impact of resveratrol on CHF exist, it could impact CHF through a number of mechanisms. As a cardioprotective agent, resveratrol acts as a potent antioxidant and antiinflammatory compound, upregulates nitric oxide synthase thereby increasing vasorelaxation, and inhibits proliferation of vascular smooth muscle cells by antagonizing endothelin-1. Resveratrol can act directly on cardiac myocytes by increasing Ca²⁻ sensitivity through an estrogen-dependent mechanism. Resveratrol has also been shown to protect against ischemic-reperfusion injury in isolated rat hearts, a response that may also be caused by triggered nitric oxide production. Nevertheless,
it becomes difficult to reconcile these presumably opposing effects (cardioprotection versus cancer protection) of resveratrol. However, studies show that resveratrol can impart antiproliferative effects that result from apoptosis in MCF-7 but not in MDA-MB-231 human breast cancer cells. Thus, there is apparently some tissue specificity of resveratrol action. Moreover, resveratrol can act as mixed antagonist/agonist depending on concentration, like other estrogenic counterparts.

As mentioned above, resveratrol is able to regulate lifespan through a highly conserved molecular mechanism. Resveratrol increases sirtuin 1 (SIRT1) activity through an allosteric interaction that results in the increase of SIRT1 affinity for both nicotinamide adenine dinucleotide and the acetylated substrate. SIRT1 is 1 of 7 mammalian sirtuin homologs of Sir2, a molecule that catalyzes nicotinamide adenine dinucleotide-dependent protein deacetylation and increases lifespan in worms and flies. The deacetylase activity of SIRT1 targets intermediates involved in the regulation of mitochondrial oxidative metabolism. Specifically, SIRT1 deacetylates PPARγ coactivator 1α (PGC-1α), a critical regulator of mitochondrial oxidative metabolism and the maintenance of glucose, lipid, and energy homeostasis, increasing its transcriptional activity. In human and rat CHF and in murine models of pressure overload, metabolic dysregulation occurs in the heart that is characterized by marked reduction in mitochondrial oxidative metabolism rates with a concomitant shift to increased glucose utilization placing PGC-1α in a key position to mediate this process. In fact, the loss of PGC-1α results in early symptoms of heart failure in mice. Moreover, no studies address the ability of resveratrol to exert cardioprotective effects through a SIRT1/PGC-1α mechanism, nor are there studies that examine how sex modifies this action. Given the estrogenic activity of resveratrol and the role of PGC-1α in cardiac disease, more studies need to be directed toward understanding the resveratrol/SIRT1/PGC-1α signaling axis and the sex dimorphisms in cardiac disease.

Concluding Remarks and Future Directions

A growing body of clinical and experimental literature addresses the interrelationship between CHF, sex, and environmental factors. However, what these data indicate is the difficulty in generating a cohesive model of cardiovascular disease progression that includes the impact of sex and environmental agents such as phytoestrogens. Significant data illustrate the widespread impact of estrogen and the mechanisms by which estrogen imparts its action. Although these mechanisms largely depend on estrogen binding to ERs, a recent study shows that ERs can be recruited in the absence of estrogen to transactivate estrogen-responsive genes. In the study by Baron et al, insulin-like growth factor 1–induced transcription is dependent on the recruitment of ERα to the activator protein 1 complex but does not require estrogen to be present. Moreover, tamoxifen (a mixed ER agonist/antagonist) or ICI 182780 (an ER antagonist), agents traditionally thought to act through ERs, negatively influence cell growth and proliferation in neonatal rat cardiomyocytes through an ER-independent mechanism. Whether these latter studies have uncovered novel signaling intermediates for estrogen-like molecules is yet to be determined, but further studies will enhance our understanding of estrogen, estrogen agonists/antagonists, and ER action. An important component of these studies will necessarily include players such as AMPK because AMPK is a major mediator of glucose uptake and oxidative metabolism, metabolic processes that become increasingly important during cardiac stress. Finally, we must incorporate into this model a complete understanding of dietary nutrients (other than lipids), especially those such as phytoestrogens that have known biological actions.

Acknowledgments

This work was supported by a National Institutes of Health grant to Dr Leinwand (HL 56510) and by a National Research Service Award (F32 HL 70509) and Mentored Research Science Development Award (K01 AR052840) from the National Institutes of Health awarded to Dr Konhilas.

Disclosures

None.

References

12. De Maria R, Guazzetti A, Recalcati F, Baroldi G, De Vita C, Camerini F. Comparison of clinical findings in idiopathic dilated cardiomyopathy in...


62. Konhilas JP, Leinwand Effects of Sex and Diet on Heart Failure 2007

63. Konhilas and Leinwand Effects of Sex and Diet on Heart Failure 2007


81. Konhilas JP, Leinwand Effects of Sex and Diet on Heart Failure 2007


88. No homemaker C, Brunner F, Wolkart G, Staber PB, Steyer E, Gonzalez FJ, Zeclinger R, Hoefler G. Myocardial dysfunction and male mortality in...


**Key Words:** cardiovascular disease  ●  diet  ●  estrogen  ●  genes  ●  sex
The Effects of Biological Sex and Diet on the Development of Heart Failure
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_Circulation_. 2007;116:2747-2759
doi: 10.1161/CIRCULATIONAHA.106.672006
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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