

Sex Hormones and Sex Chromosomes Cause Sex Differences in the Development of Cardiovascular Diseases

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Abstract—This review summarizes recent evidence concerning hormonal and sex chromosome effects in obesity, atherosclerosis, aneurysms, ischemia/reperfusion injury, and hypertension. Cardiovascular diseases occur and progress differently in the 2 sexes, because biological factors differing between the sexes have sex-specific protective and harmful effects. By comparing the 2 sexes directly, and breaking down sex into its component parts, one can discover sex-biasing protective mechanisms that might be targeted in the clinic. Gonadal hormones, especially estrogens and androgens, have long been found to account for some sex differences in cardiovascular diseases, and molecular mechanisms mediating these effects have recently been elucidated. More recently, the inherent sexual inequalities in effects of sex chromosome genes have also been implicated as contributors in animal models of cardiovascular diseases, especially a deleterious effect of the second X chromosome found in females but not in males. Hormonal and sex chromosome mechanisms interact in the sex-specific control of certain diseases, sometimes by opposing the action of the other.

Visual Overview—An online [visual overview](#) is available for this article. (*Arterioscler Thromb Vasc Biol.* 2017;37:746-756. DOI: 10.1161/ATVBAHA.116.307301.)

Key Words: androgen effects ■ atherosclerosis ■ coronary artery disease ■ estrogen effects ■ gonosomes ■ heart failure ■ obesity

Introduction: The Importance of Studying Sex Differences in Cardiovascular Diseases

Cardiovascular diseases (CVDs) manifest differently in men and women.^{1,2} The overall lifetime risk of CVD is similar in the 2 sexes, but men develop CVD earlier than women (Figure 1).^{3,4} At 55 years of age, the lifetime risk of a first incident coronary heart disease is higher in men than in women, but the risk of first incident cerebrovascular disease or heart failure is higher in women than in men.³ These sex differences suggest that biomedical principles, learned from the study of males, may not apply equally to females. CVDs should be studied in both sexes. However, separate study of both sexes is not enough. Rather, the 2 sexes must be directly compared, with the purpose of finding factors that cause sex differences and, therefore, prevent or alleviate disease in one sex more than the other. The present review summarizes limited evidence about the causes of sex differences in CVDs in humans and recent investigations of rodent models in which causal factors can be isolated and studied in a more controlled manner. Although there is no expectation that mice and other model organisms are equivalent to humans, studies of diverse organisms, which are similar to and different from humans, is the main strategy for formulating basic biological concepts that guide our understanding of human physiology. In the present case, studies of mice have recently uncovered new ideas that have yet to be applied to humans. Investigation of animals offers 2 distinct advantages. The first is the ability to independently manipulate diverse sex factors (those that cause sex

differences in physiology and disease, including hormones and numerous specific sex chromosome genes) to judge their separate effects, as well as their interactions. The second advantage is the ability to discover downstream molecular mechanisms controlled by sex factors, which might be targets for therapy. The ideas presented here include an emerging field of research based on study of animals. The new ideas will require validation and application to humans, to achieve better understanding of human physiology and disease.

Conceptual Framework

Although sex differences in CVDs are caused in part by environmental or social differences between men and women (eg, occupational hazards, habits, social stresses),⁵⁻⁷ we focus here on biological factors that are important and tractable for study in animal models.

Where do sex differences start? At the moment of conception, XX and XY zygotes differ only in their sex chromosomes. Thus, all sex differences arise from the inherent sexual inequality of these 2 chromosomes.⁸ We list here 4 classes (2 Y-linked and 2 X-linked) of sex chromosome mechanisms that could conceivably cause sex differences in phenotype.

Y Chromosome Effects

Gonadal Effects

The most important sex-differentiating effect is caused by the Y-linked gene *Sry*, which acts within gonadal primordia to cause

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Nonstandard Abbreviations and Acronyms	
AAA	abdominal aortic aneurysm
AngII	angiotensin II
ApoE	apolipoprotein
CVD	cardiovascular disease
FCG	four core genotypes mouse model
HDL	high-density lipoprotein
I/R	ischemia/reperfusion
LDL	low-density lipoprotein
Ldlr	LDL receptor
Sry	sex-determining region on the Y chromosome, the testis-determining gene
XXF	XX mouse that developed ovaries
XXM	XX mouse that developed testes
XYF	XY mouse that developed ovaries
XYM	XY mouse that developed testes
XY*	male mouse with mutant Y* chromosome, father of XY* model mice

differentiation of testes in males.⁹ Genes that are present in both sexes cause differentiation of ovaries unless *Sry* is present, so *Sry* expression is the root cause of sex differences in the type of gonads. The differentiation of testes rather than ovaries establishes a lifelong difference in the levels of gonadal hormones, especially testosterone, estradiol, and progesterone, which act directly on cardiovascular and other tissues to make them function differently in the 2 sexes. Some effects of testosterone (or its metabolite 17 β -estradiol) are permanent (organizational effects), for example, the hormone-induced male pattern of differentiation of the genitals or brain. Others are reversible (activational effects) and may last only as long as the hormone is present.¹⁰

Nongonadal Effects

Y chromosome genes act outside of the gonads to have male-specific effects. For example, *Sry* is expressed in the brain and

other tissues, especially in catecholaminergic cells and neurons, where its effects may include effects on hypertension.^{11–13} Studies of XY mice with different versions of the Y chromosome show remarkable differences in the severity of autoimmune disease.¹⁴ Effects of the Y chromosome must be specific to males, but the implied sex difference could be compensated for by other factors in females. These effects have to date been infrequently studied because historically it has been difficult to target the Y chromosome or manipulate it without also changing gonadal hormone levels. Moreover, this chromosome encodes relatively few genes, and except for *Sry*, none has been specifically implicated yet in sex differences in physiology.

X Chromosome Effects

X Gene Dosage

Most X chromosome genes do not show large sex differences in their level of expression, because one X chromosome is transcriptionally silenced in XX adult somatic cells. Thus, most X genes are expressed about equally from the single active X chromosome in both XX and XY cells. Accordingly, concepts of sexual differentiation have long ignored X chromosome genes as potential inherent causes of sex differences in phenotype. However, some genes escape inactivation and are expressed from each X chromosome so that they have higher levels in XX than in XY cells.^{15–18} Among these are a set of X chromosome genes known to be highly dosage sensitive,¹⁹ implying that one versus 2 doses of the genes are expected to cause phenotypic differences. In mice and humans, the X escapees include 2 histone demethylases, *Kdm5c* and *Kdm6a*, which are likely to have widespread effects on autosomal gene expression. Other X escapees also have fundamental effects on cell function and include the translation initiation factor *Eif2s3x* and RNA helicase *Ddx3x*, which is involved in transcriptional regulation, pre-mRNA splicing, mRNA export, cellular signaling, and viral replication. The X escapees are candidates for causing sex differences in CVD, but none has been specifically implicated to date.

X Gene Imprinting

XY cells receive a maternal imprint on X genes, but XX cells receive imprints from both parents. The inherent imbalance in parental imprinting is a potential cause of sex differences in expression of specific X genes that affect CVD phenotypes. To date, this difference in imprinting has not been specifically implicated as the cause of any sex difference in phenotype, possibly because this class of genes is not widely studied, and proving their role in sexual differentiation is experimentally complex.

In addition to the effects listed here, there may be nongenetic effects (ie, not requiring gene expression) of the X or Y chromosomes that alter the epigenetic status of the autosomes. These mechanisms are speculative and are discussed elsewhere.²⁰

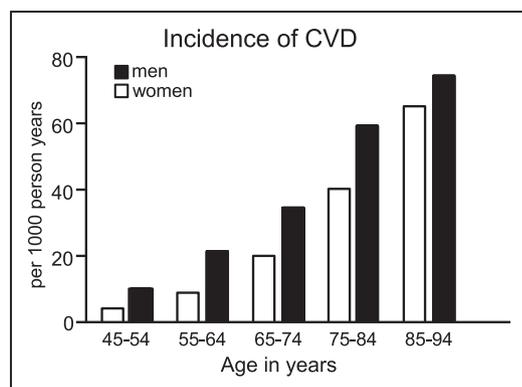


Figure 1. Incidence of cardiovascular disease (CVD) in men and women as a function of age. Data on the incidence of cardiovascular disease defined as coronary heart disease, heart failure, stroke, and intermittent claudication in women (white bars) and men (black bars) from 45 to 94 years of age. Data are derived from the Framingham Heart Study as reported by the National Heart, Lung and Blood Institute¹³⁸ and adapted from Mozaffarian et al⁴ with permission of the publisher. Copyright © 2015, American Heart Association, Inc. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

Mouse Models to Discriminate Hormonal and Sex Chromosome Effects That Cause Sex Differences

In recent years, a major goal has been to distinguish sex differences caused by gonadal hormones versus sex chromosome complement (XX versus XY). Hormonal effects are most

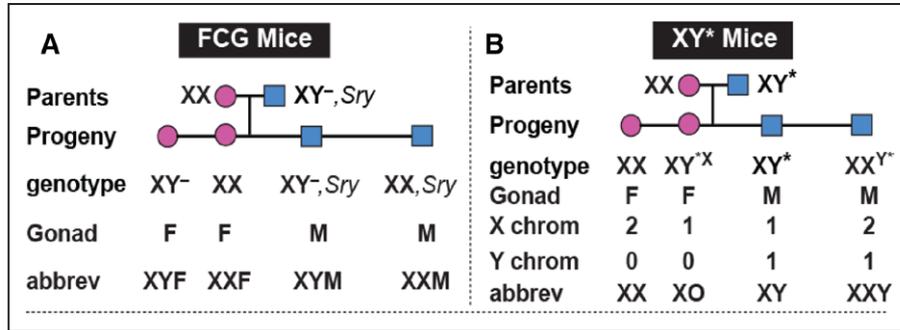


Figure 2. Two mouse models for measuring sex chromosome effects. **A**, In the four core genotypes (FCG) model, the testis-determining gene (*Sry*) is removed from Y chromosome to make the “Y-” chromosome, and an *Sry* transgene is inserted into chromosome 3. XY⁻ mice have ovaries and are called XY females (XYF) here. Mice with an *Sry* transgene have testes and are called males, XXM or XYM. **B**, The XY* model is useful for figuring out whether a difference between XX and XY is because of effects of the X chromosome or Y chromosome. It compares groups that differ in the number of X chromosomes (female mice with XX or XO and male mice with XY or XXY) or compares mice that differ in the presence/absence of Y chromosome (XO vs XY and XX vs XXY), to determine which causes XX vs XY differences. Adapted from Li et al¹⁸ with permission of the publisher. Copyright © 2014, Oxford University Press. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

often detected by manipulating hormone levels, synthesis, or action to find which hormones account for sex differences.²¹ Detecting nongonadal effects of sex chromosome complement involves manipulating the number of X and Y chromosomes, holding gonadal hormones as constant as possible. The most frequently used model is the four core genotypes (FCG) mouse model.²² In this model, *Sry* is a transgene on chromosome 3 and is not present on the Y chromosome, so that XX and XY mice can each have either testes (with *Sry*, XXM or XYM) or ovaries (without *Sry*, XXF or XYF; Figure 2). This model can discriminate between sex differences determined by gonadal type (XXM and XYM differ from XXF and XYF) versus those determined by the effects of sex chromosomes (XXF and XXM differ from XYF and XYM).

Once a sex difference is found to be caused by a sex chromosome effect, the next step is to figure out if the effect is caused by the X or Y chromosome, using the XY* mouse model (Figure 2). The Y* chromosome has a variant pseudoautosomal region that recombines abnormally with the X chromosome, producing mice with unusual complements of

sex chromosomes. For example, some progeny have a fusion of an X and Y chromosome, whereas others lack most of one X chromosome.^{23,24} The progeny are the near equivalents of XO, XX, XY, and XXY. This model allows detecting an effect of 1 versus 2 X chromosomes, by comparing XO versus XX (gonadal females) or XY versus XXY (gonadal males). One can also detect an effect of the Y chromosome by comparing XO versus XY and XX versus XXY.²⁵ Localizing the effect to the X or Y chromosome then leads to studies of candidate X or Y genes that might cause the sex chromosome effect.

Previous reviews discuss methods and interpretation of these models, including methods to avoid hormonal confounds of sex chromosome complement.^{22,26} Below, we review studies of gonadal hormone and sex chromosome influences on obesity, atherosclerosis, aneurysms, cardiac ischemia/reperfusion, and hypertension.

Obesity

Many CVDs are associated with obesity. Men and women differ in the development of obesity, and the manifestation

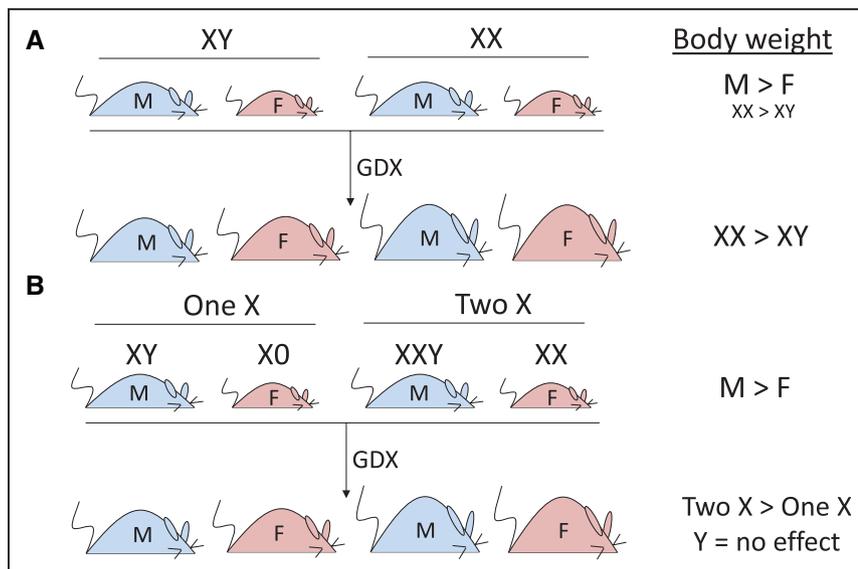


Figure 3. Summary of effects of gonadal hormones and sex chromosome complement on body weight and adiposity in four core genotypes (FCG) and XY* mice. **A**, Male mice (with testes) weigh more than female mice (with ovaries). XX mice also weigh more than XY mice, although the magnitude of effect is less than that because of gonads.¹⁷ Four weeks after gonadectomy, all 4 genotypes reach a similar weight. Thereafter, XX mice weigh more than XY mice, whether they originally had ovaries or testes. **B**, In XY* mice, males weigh more than females when gonads are intact, irrespective of sex chromosome complement. After gonadectomy, the effect of gonadal hormones is abolished, and mice with 2 X chromosomes (XXY and XX) weigh more than mice with one X chromosome (XY and XO).¹⁷

of obesity-related conditions such as hyperlipidemia, insulin resistance, and type 2 diabetes mellitus. Men generally have greater body weight than women, but the proportion of body weight as fat is greater in women.^{27–29} In the mouse, males also have greater body weight, but the degree of body fat is dependent on diet and strain. In some strains, a high-fat diet leads to similar adiposity, whereas in others one sex has greater adiposity than the other.^{30,31} Multiple mechanisms contribute to sex differences in fat storage, including ability to expand different anatomic depots (eg, greater subcutaneous fat storage in women) or ability to mobilize fat stores (eg, greater capacity for adipose tissue lipolysis in men).^{27–29} Additional sex differences include greater insulin sensitivity and higher adiponectin and leptin levels in women and female mice, and lower dietary fatty acid oxidation and greater circulating triglyceride levels in men.^{30,32} Some of the metabolic differences between men and women are reduced after menopause, possibly caused by the decline in ovarian hormones.³³ However, hormonal differences cannot fully account for all differences between men and women in lipid metabolism.³⁴

Genome-wide genetic studies in humans have identified genetic loci that influence body mass index and waist-hip-ratio specifically in one sex or to a different extent in the 2 sexes. These include loci on the autosomes and on the X chromosome.^{35,36} In the mouse, numerous genetic loci with differential effects in males and females have been described, including several loci on chromosome X.^{30,37} The mechanisms underlying sex-specific effects of genetic polymorphisms on obesity merit further study.

The use of experimental models such as the FCG and XY* mice (Figure 2) have informed us about genetic and hormonal mechanisms underlying sex differences in obesity.¹⁷ In gonad-intact FCG mice, male mice (with testes, either XX or XY) have greater body weight than female mice (with ovaries, XX and XY; Figure 3A). In addition, XX mice weigh more than XY mice of the same gonadal sex. After gonadectomy of adult mice, male–female sex differences are reduced, indicating that acute effects of gonadal hormones contribute to male–female differences in body weight. Weeks or months after gonadectomy, however, the body weight of XX mice increases more than that of XY mice, and XX mice eventually have nearly twice as much body fat.¹⁷ These results suggest that the presence of 2 X chromosomes, and/or the absence of the Y chromosome, leads to enhanced body fat. The XY* model resolves this question in favor of the X chromosome dose. Mice with 2 X chromosomes (XX or XXY) have greater body weight than mice with one X chromosome (XY or XO; Figure 3B).¹⁷ XX mice after gonadectomy also have accelerated weight gain on a high-fat diet, with increased food intake, relative to XY, during the light phase of the diurnal cycle, but with no differences in energy expenditure or locomotor activity.^{17,38} XX mice also develop more profound fatty liver, greater evidence of insulin resistance, and higher circulating cholesterol levels than XY mice when stressed with fat- or cholesterol-enriched diets.^{17,38} Thus, the number of X chromosomes has a substantial effect on obesity and related morbidities.

Localizing the sex chromosome effect to the X chromosome opens the door to identifying novel X-linked molecular determinants of sex differences in obesity. A leading

hypothesis suggests a contribution from X escapee genes, which are expressed at higher levels in XX compared with XY tissues.¹⁷ Testing this hypothesis further will involve assessing the effects on obesity when expression levels of individual X escapee genes are modulated in a controlled manner.

Atherosclerosis

Among all CVDs, coronary artery disease is the leading cause of death. Over 2 centuries ago, clinicians began to report sex differences in the prevalence of angina pectoris.³⁹ In general, women have a delay in the development of atherosclerosis compared with men, with both sexes increasing in incidence with age.⁴⁰ A recent meta-analysis suggests that increased total serum cholesterol is a significant risk factor for coronary artery disease in both sexes, with a small, but significantly stronger effect in men compared with women.⁴¹ Women have higher high-density lipoprotein (HDL) cholesterol levels and lower bile acid and cholesterol synthesis than men throughout adult life.⁴² Although sex differences in lipoprotein levels persist after menopause, endogenous sex hormone levels influence lipoprotein levels. For example, postmenopausal women have higher low-density lipoprotein (LDL) cholesterol than premenopausal women or men of the same age.^{43–45} Furthermore, ovariectomy increases LDL cholesterol levels.⁴⁶ Among individuals, the level of plasma estrone correlates with HDL cholesterol levels and correlates inversely with total cholesterol.^{47,48}

Testosterone may influence atherosclerosis in both sexes. In postmenopausal women of the Atherosclerosis Risk in Communities study, who were not taking hormone therapy, the level of sex hormone-binding globulin, which binds ≈60% of circulating testosterone, correlated with a more favorable lipid profile (lower total and LDL cholesterol and higher HDL cholesterol). Thus, relative androgen excess during menopause, rather than loss of estrogen, may adversely affect lipid status of women.⁴⁹ Conversely, testosterone has been suggested to protect males from atherosclerosis.^{50–53} Males with higher circulating testosterone concentrations had higher HDL cholesterol levels.^{54,55} In studies tracking serum lipids and lipoproteins from childhood to adulthood, levels of HDL cholesterol dropped in boys but were not changed in girls after puberty.^{56,57} These results suggest effects of endogenous testosterone during development to regulate HDL cholesterol. Moreover, the decline in testosterone levels with age⁵⁸ is associated with age-dependent increases in atherosclerosis in males,^{59–61} although other age-related factors could be causal. Serum sex hormone-binding globulin concentrations correlate inversely with atherosclerosis in men.^{62,63} These studies offer tantalizing suggestions that endogenous androgens may have opposite or divergent effects in males and females, but further research is needed.

Even more controversial than these effects of endogenous sex hormones are the effects of exogenous sex hormone therapy on coronary artery disease. In women with established coronary artery disease, administration of combined conjugated equine estrogen plus medroxyprogesterone acetate therapy increases the risk of heart disease events at 1 year.⁶⁴ In the large Women's Health Initiative, continuous treatment with conjugated equine estrogen plus medroxyprogesterone

acetate resulted in significantly increased risk of coronary artery events and stroke.^{65,66} Finally, in women with hysterectomies administered unopposed estrogen (specifically, oral conjugated equine estrogen, 0.625 mg/d) for the prevention of chronic postmenopausal conditions, there were no benefits on coronary heart disease, with an increased risk for venous thromboembolism and stroke.⁶⁷ It is important to note that the number of risk factors present in the study population and other differences in study design (eg, use of statins) may have influenced the cardiovascular outcomes of these studies. Recent results from the ELITE (Early versus Late Intervention Trial with Estradiol) highlights the potential importance of timing of initiation of 17 β -estradiol therapy relative to menopause.⁶⁸ Oral 17 β -estradiol therapy was associated with less progression of subclinical atherosclerosis when therapy was initiated within 6 years after menopause but not when therapy was initiated ≥ 10 years after menopause. These differential effects of estradiol therapy might be related to changes in estrogen receptor expression in pivotal target tissues such as the vasculature. Current guidelines continue to caution against use of postmenopausal hormone therapy to prevent cardiovascular events in women. However, given the conflicting conclusions reached from these various studies, an understanding of cardiovascular risk in women requires further research on hormone treatment in terms of type and delivery method of estrogen and progesterone, as well as the timing and duration of treatment.

Inbred mouse strains are valuable for varying sex and other variables when genetic background is held constant. Mice and humans differ in lipid profile, with mice carrying the majority of blood cholesterol in HDL particles, whereas humans have much greater levels of LDL cholesterol.⁶⁹ These species differences can be diminished by using mice with elevated LDL or very-low-density lipoprotein cholesterol levels caused by genetic manipulations (eg, LDL receptor [*Ldlr*] or apolipoprotein E [ApoE] deficiency) or diet.^{69,70} Sex differences in atherosclerotic lesions have been examined in mouse models. In ApoE-deficient mice, some reports describe larger aortic root lesions in female compared with male mice,^{71,72} but others suggest that this is true only in younger mice, with older ApoE-deficient mice showing lesions of similar size and composition.⁷³ Mouse models have demonstrated a relatively consistent protective effect of estrogens to reduce atherosclerosis.⁷⁴ In *Ldlr*-deficient and ApoE-deficient female mice, ovariectomy increases atherosclerosis, which is reversed by administration of 17 β -estradiol.⁷⁵⁻⁷⁷ Interestingly, protective effects of estradiol are independent of changes in plasma cholesterol and do not occur in mice lacking estrogen receptor- α globally or in endothelial cells.⁷⁸⁻⁸⁰ Estradiol may be protective because of its effects to increase endothelial NO,^{81,82} prostacyclin,^{83,84} and macrophage cholesterol efflux⁸⁵ and to reduce macrophage inflammation⁸⁶⁻⁸⁸ and smooth muscle cell proliferation.^{89,90} Nevertheless, a great deal more information is needed to understand estrogen effects on lesion development, progression, and regression.

Similar to exogenous estrogen, there is considerable controversy over the benefits of testosterone replacement therapy for aging males.⁹¹ In 2015, the US Food and Drug Administration issued a caution on the use of testosterone products in aging males based on increased risk of heart attack

and stroke. The caution was based on a review of 5 retrospective cohort studies and 2 meta-analyses of controlled testosterone trials. Although results were not entirely consistent across these studies, the US Food and Drug Administration concluded that there was sufficient concern that testosterone administration may increase adverse cardiovascular outcomes. To date, however, there have been no randomized controlled trials to evaluate cardiovascular outcomes with testosterone use.

Studies of animal models of atherosclerosis indicate that testosterone regulates levels of apolipoprotein B-containing lipoproteins,⁹² HDL cholesterol,^{93,94} vascular cell adhesion molecule-1,⁹⁵ foam cell formation,⁹⁶ and vascular calcification.⁹⁷ However, some of these studies were performed in genetically manipulated mice (eg, ApoE-deficient mice) with lower HDL cholesterol raising concern about their applicability to testosterone regulation of these lipoproteins in humans.

In addition to sex hormones, sex chromosomes have been linked to the regulation of serum lipid profiles and coronary artery disease. Polysomy of the Y chromosome (primarily 47, XYY karyotype) is associated with increased cardiovascular mortality.⁹⁸ Europeans with a common inherited haplotype of the Y chromosome have a 50% increased risk of coronary artery disease that is associated with an altered immune response.⁹⁹ In contrast, women with monosomy X (Turner syndrome) have an atherogenic lipid profile (increased LDL cholesterol level and particle size)¹⁰⁰ and exhibit an increased risk for ischemic heart disease.¹⁰¹ Most sex chromosome aneuploidy conditions (eg, XO and XXY) in humans, however, involve concomitant changes in gonadal hormone levels, making it difficult to separate sex chromosome and hormonal effects. Studies in the FCG mouse model have revealed both gonadal sex and sex chromosome contributions to the regulation of serum lipid profiles, with particularly robust effects of the X chromosome dosage on HDL cholesterol levels.³⁸ Further studies will be required to determine the mechanisms by which sex chromosome complement influences lipid profiles and coronary artery risk.

Aneurysms

Sex is the largest nonmodifiable risk factor for abdominal aortic aneurysms (AAAs), with males having 4- to 10-fold increased risk of AAA development.^{102,103} This pronounced sex difference has resulted in current screening guidelines for AAA diagnosis that only apply to males.^{104,105} The lack of screening in women is a serious concern. Although the prevalence of AAAs is much lower in women, the rate of expansion and propensity to rupture is more aggressive in females.¹⁰⁶⁻¹⁰⁸ Recent studies estimate that AAA deaths in women (45% of all prevalent cases) are higher than would be expected based on an overall lower incidence of AAA in females.¹⁰⁹ Regardless, there are no effective therapies that slow the progression of AAAs in either sex, much less therapies that address potential sex differences in AAA growth mechanisms.

Studies of animal models have increased understanding of mechanisms, mostly hormonal, underlying sex differences in AAAs. In general, endogenous and exogenous estrogens have protective effects on experimentally induced AAAs, with results differing dependent on the model under study. In elastase-induced AAAs, estrogen receptor- α expression in aorta

is greater in females than in males,¹¹⁰ and estrogen synthesis in ovaries and nonovarian tissues contributes to protection of female mice.¹¹¹ When AAAs are induced by infusion of angiotensin II (AngII) in female ApoE-deficient mice, ovariectomy has no effect on AAAs,⁹⁴ but treatment of males with estrogen attenuates AAA development.¹¹² Moreover, administration of exogenous 17 β -estradiol to ovariectomized females abolishes progressive increases in abdominal aortic diameter and AAA size.¹¹³ Collectively, these results support a potential effect of exogenous estrogens to reduce experimental AAA expansion.

Testosterone has also been implicated as a contributor to experimental AAA formation and progression in male and female mice. In the AngII model of AAAs, castration of male mice markedly reduces AAA formation, which is restored by administration of dihydrotestosterone.^{94,114} These effects are attributed to testosterone's effect to increase expression of abdominal aortic type 1a angiotensin receptor.¹¹⁴ Notably, whereas female hypercholesterolemic mice are resistant to AngII-induced AAAs, adult *Ldlr*^{-/-} female mice previously treated with testosterone as neonates are highly susceptible to AngII-induced AAAs even though they have low levels of testosterone in adulthood.¹¹⁵ Finally, castration of adult male *ApoE*^{-/-} mice, after an AAA has formed from AngII infusion, halts progressive aortic dilations caused by continued AngII infusions.¹¹⁶ Although evidence from animal models suggests a clear role for testosterone to promote AAAs, there is little known about a role for endogenous or exogenous testosterone on human AAAs.

The interesting dichotomy between males and females in the duration of testosterone exposure required to increase AAA susceptibility suggests that other mechanisms, potentially sex chromosomes, contribute to sex differences in AngII-induced AAAs. This idea is supported by a recent comparison of *Ldlr*^{-/-} XX female versus XY female mice from the FCG model infused with AngII to induce AAA formation.¹¹⁷ XY females had much greater AAA formation than XX females, an effect that was independent of ovarian hormone levels, and exacerbated by treatment with androgens. As in other measures of CVDs, both sex chromosome complement and gonadal hormones interact to affect disease outcomes. In this case, a male complement of sex chromosomes (XY) and male gonadal hormones may synergize in their effects to cause sex differences.

Cardiac Ischemia/Reperfusion Injury

Women before menopause have lower risk of ischemic heart disease than age-matched men.¹¹⁸ The lower incidence of coronary artery disease in women during reproductive age is believed to be caused in part by ovarian hormones because loss of ovaries in young women increases coronary artery disease.¹¹⁹ Similarly, when mice receive ischemia/reperfusion (I/R) injury to mimic heart attack, infarct size in ovariectomized mice is larger than that in gonadally intact mice.¹²⁰ Among the gonadal sex hormones, estradiol is most convincingly reported to be protective in animal studies of myocardial I/R injury.¹²¹ Estradiol pretreatment reduces the infarct size in ovariectomized mice¹²² and in male mice subjected to in vivo I/R injury.¹²³

If estrogens were the only factors accounting sex differences in ischemic heart disease in humans, one might expect an increase of disease after menopause in women because of the reduction in plasma estrogens and that estrogenic hormone replacement therapy might help prevent heart disease. The Women's Health Initiative study, however, failed to find that hormone replacement therapy reduced the risk of ischemic heart disease of postmenopausal women.^{64,65} Moreover, a recent large population-based prospective study found only a minor increase in the risk of ischemic heart disease after menopause at 55 to 65 years of age. Indeed, the risk of ischemic heart disease increases in both men and women with age until the age of 65–69 years and more rapidly up to 95 years of age, and the sex difference in incidence persists throughout life with perhaps a modest reduction in the size of the sex difference after 85 years of age.^{124,125} This pattern suggests that ovarian hormones do not alone account for the sex differences, and we should look for factors in males that make them different from females and other factors in females that contribute to their incidence of disease. Recent studies of gonadectomized mice with altered sex chromosomes (FCG and XY* models, Figure 2) found sex differences in I/R caused by the number and type of sex chromosomes.¹⁸ Gonadectomized adult mice with 2 X chromosomes (XX or XXY) have \approx 50% larger infarct size, compared with mice with one X chromosome (XY or XO). The presence versus absence of the Y chromosome had no effect, so Y genes do not contribute to the sex difference in I/R injury under these conditions. Thus, the presence of a second X chromosome has deleterious effects both on adiposity¹⁷ (reviewed above) and on I/R injury. However, the effects on I/R injury are not secondary to greater adiposity because they exist before the effects on adiposity are evident.¹²⁶ Again, as for effects on adiposity, the X escapee genes are top candidates for causing these effects because of their higher expressed dose in mice with 2 X chromosomes.

The X chromosome effect in mice seems paradoxical because it goes in the wrong direction to explain the reduced susceptibility of women to I/R injury. In gonadectomized female mice, the second X chromosome promotes rather than prevents injury. This observation nevertheless has potential clinical significance in humans because female-specific factors (eg, estrogens and the second X chromosome) may interact with one another such that the protective effect of estradiol is mitigated by the presence of a second X chromosome. I/R disease occurs mostly in older women, when estrogen levels are low. Thus, women may be protected by estrogens early in life, but after menopause, they may be more susceptible to I/R injury especially because of the deleterious effects of the second X chromosome. These ideas, stemming from animal research, will be easier to test in humans once the X gene(s) causing the effect have been discovered in mice.

Hypertension

According to the World Health Organization, hypertension is a major cause of CVD leading to death throughout the world.¹²⁷ The onset of hypertension occurs earlier in men than in women in diverse human populations. The finding of

male susceptibility and female resilience is mirrored in the vast majority of animal models of hypertension, regardless of whether it is induced or genetic.¹²⁸

Many studies suggest that estradiol is a major factor in female resilience to developing hypertension. The prevalence of hypertension is greater in women with ovarian hormone deficiency (eg, premature ovarian failure or ovariectomy before menopause) and in postmenopausal women compared with age-matched premenopausal women. Furthermore, animal studies show that blood pressure is increased in females that are ovariectomized or lack estrogen receptor- α .¹²⁸

Animal models of testosterone deficiency (eg, castration) suggest that the presence of testosterone can contribute to male susceptibility to hypertension, and anabolic steroid use in men is associated with increased blood pressure. However, the prevalence of hypertension is greater in men with clinically low testosterone levels.¹²⁸ Thus, more investigation into the effects of sex steroids in men is necessary to gain a clearer understanding of their role.

One exciting area of sex difference research in hypertension involves the immune system. Whereas male mice have higher blood pressure than females after AngII infusion, this sex difference disappears in mice deficient in T cells.^{129,130} Adoptive transfer of male T cells into T-cell-deficient mice exacerbates hypertension in male but not female recipient mice, indicating that the sex of the host plays a key role in resilience and susceptibility to immune modulation of hypertension.¹²⁹ Furthermore, T-cell modulation of blood pressure depends on the sex of the donor because adoptive transfer of male but not female T cells can exacerbate the degree of AngII-induced hypertension in the male host.¹³⁰

Experiments using FCG mice have enabled dissection of the role of the sex chromosomes from the gonadal hormones. The magnitude of hypertension induced by AngII infusion is greater in gonadectomized XX mice compared with the XY, regardless of whether the mice were born with ovaries or testes.¹³¹ Further studies using this model suggest that females with intact ovaries are protected from hypertension because of vasodilatory mechanisms mediated by endothelium-derived hyperpolarizing factors and the AngII receptor. Although endothelium-derived hyperpolarizing factors only require estradiol, AngII receptor-mediated vasodilation requires both estradiol and the XX sex chromosome complement.¹³² Thus, ovarian hormones and sex chromosomes seem to have complex effects on susceptibility to hypertension, similar to I/R injury.

Although the risk of CVD increases with higher blood pressures in both sexes, the risk of developing CVD is greater in men than women at equivalent age and blood pressure (Figure 4).^{133,134} Understanding why men are at greater risk or why women are at lower risk may lead to the development of novel therapeutics that would be beneficial for treating or preventing CVD in both men and women. Most animal studies of hypertension-associated CVD including ischemic heart disease, stroke, heart failure, atrial fibrillation, and cardiac sudden death have been conducted in males. In the few that have directly compared markers of CVD end-organ damage between males and females, most show that males exhibit greater injury than females.

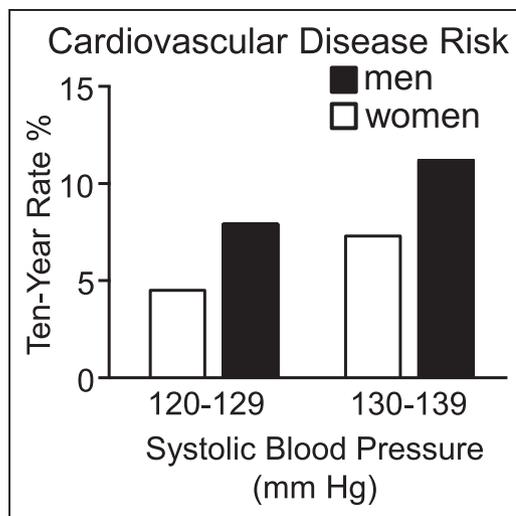


Figure 4. Cardiovascular disease risk in middle-aged men and women as a function of blood pressure. Shown are data on estimated 10-y rates of cardiovascular disease risk defined as a composite of coronary heart disease, cerebrovascular events, peripheral artery disease, and heart failure in normotensive (systolic blood pressure: 120–129 mm Hg) and prehypertensive (systolic blood pressure: 130–139 mm Hg) women (white bar) and men (black bar) 50 to 54 y of age who were nonsmokers and did not have diabetes mellitus. Data are derived from the Framingham Heart Study as reported by D’Agostino et al¹³⁴ and adapted from Mozaffarian et al⁴ with permission of the publisher. Copyright © 2015, American Heart Association, Inc. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

For those studies that investigated the cause of the sex differences, estradiol was shown to be a major contributor to female protection. For example, in a study of endothelial damage in the spontaneously hypertensive rat, estradiol protected the vasculature by increasing the generation of the vasodilator NO in the endothelium and reduced superoxide anion concentrations by inhibiting AngII receptor-mediated production of superoxide anion via NAD(P)H oxidase.¹³⁵ No studies to date have rigorously examined the cause of sex differences in the direct relationship between blood pressure and magnitude of injury in models of CVD or in the mechanisms underlying these sex differences. Thus, we do not understand why the risk of developing CVD is far greater in men than women at equivalent blood pressures.

Conclusions: The Future of Sex Differences in CVD

The studies summarized here reveal pervasive sex differences in CVDs and document diverse mechanisms that contribute to sex differences in CVD. To understand the mechanisms underlying sex differences, the biological variable of sex must be broken down into its component parts. At the outset, we listed 4 categories of sex-biasing mechanisms: gonadal (hormone-mediated) effects of Sry, nongonadal effects of Y genes, X gene dosage, and imprinting of X genes. There is strong support that sex differences are created by some of these mechanisms, especially effects of gonadal hormones, but others are known (sex chromosome effects) but have not yet been

rigorously explained by dosage or imprinting effects of specific X or Y genes. The vast majority of studies, in humans and animal models, have focused on the role of gonadal hormones. As reviewed within specific sections above, estrogens are the most frequently studied factors and have protective effects in all types of CVDs discussed here. The relative dearth of studies of androgens points to an important area for further research. The potential importance of sex chromosome complement, especially X chromosome number, has only recently been discovered in research on mice. However, the X genes involved, and their sites and mechanisms of action, are completely unknown. Moreover, hormonal and sex chromosome mechanisms clearly influence the same disease outcomes, sometimes in opposite directions,¹³⁶ but the mechanistic nature of this interaction is completely unstudied. Finally, the sex-biased protective mechanisms of sex hormones and sex chromosome genes, discovered by manipulating specific sex-biasing factors in animal research, have yet to be fully translated to the clinic. Evidence suggests already that the effectiveness of specific drug therapies may depend on sex or levels of sex-biasing factors such as estrogens.¹³⁷ We anticipate a rich series of studies in the near future that will make progress on these exciting questions.

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Disclosures

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References

- US National Institute of Medicine Committee on Understanding the Biology of Sex and Gender Disorders. *Exploring the Biological Contributions to Human Health: Does Sex Matter?* Washington, DC: National Academy Press; 2001.
- Maric-Bilkcan C, Arnold AP, Taylor DA, Dwinell M, Howlett SE, Wenger N, Reckelhoff JF, Sandberg K, Churchill G, Levin E, Lundberg MS. Report of the National Heart, Lung, and Blood Institute Working Group on sex differences research in cardiovascular disease: scientific questions and challenges. *Hypertension*. 2016;67:802–807. doi: 10.1161/HYPERTENSIONAHA.115.06967.
- Leening MJ, Ferket BS, Steyerberg EW, Kavousi M, Deckers JW, Nieboer D, Heeringa J, Portegies ML, Hofman A, Ikram MA, Hunink MG, Franco OH, Stricker BH, Wittteman JC, Roos-Hesselink JW. Sex differences in lifetime risk and first manifestation of cardiovascular disease: prospective population based cohort study. *BMJ*. 2014;349:g5992.
- Mozaffarian D, Benjamin EJ, Go AS, et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2015 update: a report from the American Heart Association. *Circulation*. 2015;131:e29–322. doi: 10.1161/CIR.000000000000152.
- Shi A, Tao Z, Wei P, Zhao J. Epidemiological aspects of heart diseases. *Exp Ther Med*. 2016;12:1645–1650. doi: 10.3892/etm.2016.3541.
- Puckrein GA, Egan BM, Howard G. Social and medical determinants of cardiometabolic health: the big picture. *Ethn Dis*. 2015;25:521–524. doi: 10.18865/ed.25.4.521.
- Pickering TG. The effects of environmental and lifestyle factors on blood pressure and the intermediary role of the sympathetic nervous system. *J Hum Hypertens*. 1997;11(suppl 1):S9–S18.
- Arnold AP. The end of gonad-centric sex determination in mammals. *Trends Genet*. 2012;28:55–61. doi: 10.1016/j.tig.2011.10.004.
- Eggers S, Ohnesorg T, Sinclair A. Genetic regulation of mammalian gonad development. *Nat Rev Endocrinol*. 2014;10:673–683. doi: 10.1038/nrendo.2014.163.
- Arnold AP. The organizational-activational hypothesis as the foundation for a unified theory of sexual differentiation of all mammalian tissues. *Horm Behav*. 2009;55:570–578. doi: 10.1016/j.yhbeh.2009.03.011.
- Dewing P, Chiang CW, Sinchak K, Sim H, Fernagut PO, Kelly S, Chesselet MF, Micevych PE, Albrecht KH, Harley VR, Vilain E. Direct regulation of adult brain function by the male-specific factor SRY. *Curr Biol*. 2006;16:415–420. doi: 10.1016/j.cub.2006.01.017.
- Loke H, Harley V, Lee J. Biological factors underlying sex differences in neurological disorders. *Int J Biochem Cell Biol*. 2015;65:139–150. doi: 10.1016/j.biocel.2015.05.024.
- Ely D, Underwood A, Dunphy G, Boehme S, Turner M, Milsted A. Review of the Y chromosome, Sry and hypotension. *Steroids*. 2010;75:747–753. doi: 10.1016/j.steroids.2009.10.015.
- Case LK, Teuscher C. Y genetic variation and phenotypic diversity in health and disease. *Biol Sex Differ*. 2015;6:6. doi: 10.1186/s13293-015-0024-z.
- Disteche CM. Dosage compensation of the sex chromosomes. *Annu Rev Genet*. 2012;46:537–560. doi: 10.1146/annurev-genet-110711-155454.
- Balaton BP, Brown CJ. Escape artists of the X chromosome. *Trends Genet*. 2016;32:348–359. doi: 10.1016/j.tig.2016.03.007.
- Chen X, McClusky R, Chen J, Beaven SW, Tontonoz P, Arnold AP, Reue K. The number of x chromosomes causes sex differences in adiposity in mice. *PLoS Genet*. 2012;8:e1002709. doi: 10.1371/journal.pgen.1002709.
- Li J, Chen X, McClusky R, Ruiz-Sundstrom M, Itoh Y, Umar S, Arnold AP, Eghbali M. The number of X chromosomes influences protection from cardiac ischaemia/reperfusion injury in mice: one X is better than two. *Cardiovasc Res*. 2014;102:375–384. doi: 10.1093/cvr/cvu064.
- Bellott DW, Hughes JF, Skaletsky H, et al. Mammalian Y chromosomes retain widely expressed dosage-sensitive regulators. *Nature*. 2014;508:494–499. doi: 10.1038/nature13206.
- Wijchers PJ, Festenstein RJ. Epigenetic regulation of autosomal gene expression by sex chromosomes. *Trends Genet*. 2011;27:132–140. doi: 10.1016/j.tig.2011.01.004.
- Becker JB, Arnold AP, Berkley KJ, Blaustein JD, Eckel LA, Hampson E, Herman JP, Marts S, Sadee W, Steiner M, Taylor J, Young E. Strategies and methods for research on sex differences in brain and behavior. *Endocrinology*. 2005;146:1650–1673. doi: 10.1210/en.2004-1142.
- Arnold AP. Conceptual frameworks and mouse models for studying sex differences in physiology and disease: why compensation changes the game. *Exp Neurol*. 2014;259:2–9. doi: 10.1016/j.expneurol.2014.01.021.
- Eicher EM, Hale DW, Hunt PA, Lee BK, Tucker PK, King TR, Eppig JT, Washburn LL. The mouse Y* chromosome involves a complex rearrangement, including interstitial positioning of the pseudoautosomal region. *Cytogenet Cell Genet*. 1991;57:221–230.
- Burgoyne PS, Mahadevaiah SK, Perry J, Palmer SJ, Ashworth A. The Y* rearrangement in mice: new insights into a perplexing PAR. *Cytogenet Cell Genet*. 1998;80:37–40.
- Chen X, McClusky R, Itoh Y, Reue K, Arnold AP. X and Y chromosome complement influence adiposity and metabolism in mice. *Endocrinology*. 2013;154:1092–1104. doi: 10.1210/en.2012-2098.
- Arnold AP, Chen X. What does the “four core genotypes” mouse model tell us about sex differences in the brain and other tissues? *Front Neuroendocrinol*. 2009;30:1–9. doi: 10.1016/j.yfrne.2008.11.001.
- Fried SK, Lee MJ, Karastergiou K. Shaping fat distribution: new insights into the molecular determinants of depot- and sex-dependent adipose biology. *Obesity (Silver Spring)*. 2015;23:1345–1352. doi: 10.1002/oby.21133.
- Palmer BF, Clegg DJ. The sexual dimorphism of obesity. *Mol Cell Endocrinol*. 2015;402:113–119. doi: 10.1016/j.mce.2014.11.029.
- Mauvais-Jarvis F. Sex differences in metabolic homeostasis, diabetes, and obesity. *Biol Sex Differ*. 2015;6:14. doi: 10.1186/s13293-015-0033-y.
- Parks BW, Sallam T, Mehrabian M, et al. Genetic architecture of insulin resistance in the mouse. *Cell Metab*. 2015;21:334–346. doi: 10.1016/j.cmet.2015.01.002.
- Parks BW, Nam E, Org E, et al. Genetic control of obesity and gut microbiota composition in response to high-fat, high-sucrose diet in mice. *Cell Metab*. 2013;17:141–152. doi: 10.1016/j.cmet.2012.12.007.
- Santosa S, Jensen MD. The sexual dimorphism of lipid kinetics in humans. *Front Endocrinol (Lausanne)*. 2015;6:103. doi: 10.3389/fendo.2015.00103.

33. Law J, Bloor I, Budge H, Symonds ME. The influence of sex steroids on adipose tissue growth and function. *Horm Mol Biol Clin Investig.* 2014;19:13–24. doi: 10.1515/hmbci-2014-0015.
34. Wang X, Magkos F, Mittendorfer B. Sex differences in lipid and lipoprotein metabolism: it's not just about sex hormones. *J Clin Endocrinol Metab.* 2011;96:885–893. doi: 10.1210/jc.2010-2061.
35. Winkler TW, Justice AE, Graff M, et al; CHARGE Consortium; DIAGRAM Consortium; GLGC Consortium; Global-BPGen Consortium; ICBP Consortium; MAGIC Consortium. The influence of age and sex on genetic associations with adult body size and shape: a large-scale genome-wide interaction study. *PLoS Genet.* 2015;11:e1005378. doi: 10.1371/journal.pgen.1005378.
36. Sung YJ, Pérusse L, Sarzynski MA, et al. Genome-wide association studies suggest sex-specific loci associated with abdominal and visceral fat. *Int J Obes (Lond).* 2016;40:662–674. doi: 10.1038/ijo.2015.217.
37. Bennett BJ, Davis RC, Civelek M, et al. Genetic architecture of atherosclerosis in mice: a systems genetics analysis of common inbred strains. *PLoS Genet.* 2015;11:e1005711. doi: 10.1371/journal.pgen.1005711.
38. Link JC, Chen X, Prien C, Borja MS, Hammerson B, Oda MN, Arnold AP, Reue K. Increased high-density lipoprotein cholesterol levels in mice with XX versus XY sex chromosomes. *Arterioscler Thromb Vasc Biol.* 2015;35:1778–1786. doi: 10.1161/ATVBAHA.115.305460.
39. Heberden W. Commentaries on the history and cure of diseases. In: Willis FA, Keys TE, eds. *Cardiac Classics*. St. Louis: C.V. Mosby; 1941:224.
40. McGill HC, Stern MP. Sex and atherosclerosis. *Atheroscler Res.* 1979;4:157–242.
41. Peters SA, Singhateh Y, Mackay D, Huxley RR, Woodward M. Total cholesterol as a risk factor for coronary heart disease and stroke in women compared with men: a systematic review and meta-analysis. *Atherosclerosis.* 2016;248:123–131. doi: 10.1016/j.atherosclerosis.2016.03.016.
42. Gálman C, Angelin B, Rudling M. Pronounced variation in bile acid synthesis in humans is related to gender, hypertriglyceridaemia and circulating levels of fibroblast growth factor 19. *J Intern Med.* 2011;270:580–588. doi: 10.1111/j.1365-2796.2011.02466.x.
43. Middelberg RP, Spector TD, Swaminathan R, Snieder H. Genetic and environmental influences on lipids, lipoproteins, and apolipoproteins: effects of menopause. *Arterioscler Thromb Vasc Biol.* 2002;22:1142–1147.
44. Kannel WB, Hjortland MC, McNamara PM, Gordon T. Menopause and risk of cardiovascular disease: the Framingham study. *Ann Intern Med.* 1976;85:447–452.
45. Guthrie JR, Taffe JR, Lehert P, Burger HG, Dennerstein L. Association between hormonal changes at menopause and the risk of a coronary event: a longitudinal study. *Menopause.* 2004;11:315–322.
46. Oliver MF, Boyd GS. Effect of bilateral ovariectomy on coronary-artery disease and serum-lipid levels. *Lancet.* 1959;2:690–694.
47. Cauley JA, Gutai JP, Glynn NW, Paternostro-Bayles M, Cottington E, Kuller LH. Serum estrone concentrations and coronary artery disease in postmenopausal women. *Arterioscler Thromb.* 1994;14:14–18.
48. Kumagai S, Kai Y, Sasaki H. Relationship between insulin resistance, sex hormones and sex hormone-binding globulin in the serum lipid and lipoprotein profiles of Japanese postmenopausal women. *J Atheroscler Thromb.* 2001;8:14–20.
49. Mudali S, Dobs AS, Ding J, Cauley JA, Szklo M, Golden SH; Atherosclerosis Risk in Communities Study. Endogenous postmenopausal hormones and serum lipids: the atherosclerosis risk in communities study. *J Clin Endocrinol Metab.* 2005;90:1202–1209. doi: 10.1210/jc.2004-0744.
50. Farias JM, Tinetti M, Khoury M, Umpierrez GE. Low testosterone concentration and atherosclerotic disease markers in male patients with type 2 diabetes. *J Clin Endocrinol Metab.* 2014;99:4698–4703. doi: 10.1210/jc.2014-2585.
51. Shores MM, Matsumoto AM. Testosterone, aging and survival: biomarker or deficiency. *Curr Opin Endocrinol Diabetes Obes.* 2014;21:209–216. doi: 10.1097/MED.0000000000000057.
52. Muller M, van den Beld AW, Bots ML, Grobbee DE, Lamberts SW, van der Schouw YT. Endogenous sex hormones and progression of carotid atherosclerosis in elderly men. *Circulation.* 2004;109:2074–2079. doi: 10.1161/01.CIR.0000125854.51637.06.
53. Lee WC, Kim MT, Ko KT, Lee WK, Kim SY, Kim HY, Yang DY. Relationship between serum testosterone and cardiovascular disease risk determined using the Framingham risk score in male patients with sexual dysfunction. *World J Mens Health.* 2014;32:139–144. doi: 10.5534/wjmh.2014.32.3.139.
54. Gutai J, LaPorte R, Kuller L, Dai W, Falvo-Gerard L, Caggiula A. Plasma testosterone, high density lipoprotein cholesterol and other lipoprotein fractions. *Am J Cardiol.* 1981;48:897–902.
55. Heller RF, Miller NE, Lewis B, Vermeulen A, Fairney A, James VH, Swan AV. Associations between sex hormones, thyroid hormones and lipoproteins. *Clin Sci (Lond).* 1981;61:649–651.
56. Webber LS, Srinivasan SR, Wattigney WA, Berenson GS. Tracking of serum lipids and lipoproteins from childhood to adulthood. The Bogalusa Heart Study. *Am J Epidemiol.* 1991;133:884–899.
57. Eissa MA, Mihalopoulos NL, Holubkov R, Dai S, Labarthe DR. Changes in fasting lipids during puberty. *J Pediatr.* 2016;170:199–205. doi: 10.1016/j.jpeds.2015.11.018.
58. Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR; Baltimore Longitudinal Study of Aging. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. *J Clin Endocrinol Metab.* 2001;86:724–731. doi: 10.1210/jcem.86.2.7219.
59. Araujo AB, Dixon JM, Suarez EA, Murad MH, Guey LT, Wittert GA. Clinical review: endogenous testosterone and mortality in men: a systematic review and meta-analysis. *J Clin Endocrinol Metab.* 2011;96:3007–3019. doi: 10.1210/jc.2011-1137.
60. Corona G, Monami M, Boddi V, Rastrelli G, Melani C, Balzi D, Sforza A, Forti G, Mannucci E, Maggi M. Pulse pressure independently predicts major cardiovascular events in younger but not in older subjects with erectile dysfunction. *J Sex Med.* 2011;8:247–254. doi: 10.1111/j.1743-6109.2010.01966.x.
61. Ruige JB, Mahmoud AM, De Bacquer D, Kaufman JM. Endogenous testosterone and cardiovascular disease in healthy men: a meta-analysis. *Heart.* 2011;97:870–875. doi: 10.1136/hrt.2010.210757.
62. Hak AE, Witteman JC, de Jong FH, Geerlings MI, Hofman A, Pols HA. Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam study. *J Clin Endocrinol Metab.* 2002;87:3632–3639. doi: 10.1210/jcem.87.8.8762.
63. Rosano GM, Sheiban I, Massaro R, Pagnotta P, Marazzi G, Vitale C, Mercurio G, Volterrani M, Aversa A, Fini M. Low testosterone levels are associated with coronary artery disease in male patients with angina. *Int J Impot Res.* 2007;19:176–182. doi: 10.1038/sj.ijir.3901504.
64. Hulley S, Grady D, Bush D, Furberg C, Herrington D, Riggs B, Vittinghoff E. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *JAMA.* 1998;280:605–613.
65. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J; Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA.* 2002;288:321–333.
66. Manson JE, Hsia J, Johnson KC, Rossouw JE, Assaf AR, Lasser NL, Trevisan M, Black HR, Heckbert SR, Detrano R, Strickland OL, Wong ND, Crouse JR, Stein E, Cushman M; Women's Health Initiative Investigators. Estrogen plus progestin and the risk of coronary heart disease. *N Engl J Med.* 2003;349:523–534. doi: 10.1056/NEJMoa030808.
67. US Preventative Services Task Force. Hormone therapy for the prevention of chronic conditions in postmenopausal women: recommendations from the US Preventative Services Task Force. *Ann Intern Med* 2005;142:855–860.
68. Hodis HN, Mack WJ, Henderson VW, Shoupe D, Budoff MJ, Hwang-Levine J, Li Y, Feng M, Dustin L, Kono N, Stanczyk FZ, Selzer RH, Azen SP; ELITE Research Group. Vascular effects of early versus late postmenopausal treatment with estradiol. *N Engl J Med.* 2016;374:1221–1231. doi: 10.1056/NEJMoa1505241.
69. Getz GS, Reardon CA. Animal models of atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2012;32:1104–1115. doi: 10.1161/ATVBAHA.111.237693.
70. Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: a dynamic balance. *Nat Rev Immunol.* 2013;13:709–721. doi: 10.1038/nri3520.
71. Smith DD, Tan X, Tawfik O, Milne G, Stechschulte DJ, Dileepan KN. Increased aortic atherosclerotic plaque development in female apolipoprotein E-null mice is associated with elevated thromboxane A2 and decreased prostacyclin production. *J Physiol Pharmacol.* 2010;61:309–316.
72. Tangirala RK, Rubin EM, Palinski W. Quantitation of atherosclerosis in murine models: correlation between lesions in the aortic origin and in the entire aorta, and differences in the extent of lesions between sexes in LDL receptor-deficient and apolipoprotein E-deficient mice. *J Lipid Res.* 1995;36:2320–2328.

73. Caligiuri G, Nicoletti A, Zhou X, Törnberg I, Hansson GK. Effects of sex and age on atherosclerosis and autoimmunity in apoE-deficient mice. *Atherosclerosis*. 1999;145:301–308.
74. Hodgins JB, Maeda N. Minireview: estrogen and mouse models of atherosclerosis. *Endocrinology*. 2002;143:4495–4501. doi: 10.1210/endo.143.12.9995.
75. Marsh MM, Walker VR, Curtiss LK, Banka CL. Protection against atherosclerosis by estrogen is independent of plasma cholesterol levels in LDL receptor-deficient mice. *J Lipid Res*. 1999;40:893–900.
76. Elhage R, Arnal JF, Pieraggi MT, Duverger N, Fiévet C, Faye JC, Bayard F. 17 beta-estradiol prevents fatty streak formation in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*. 1997;17:2679–2684.
77. Bourassa PA, Milos PM, Gaynor BJ, Breslow JL, Aiello RJ. Estrogen reduces atherosclerotic lesion development in apolipoprotein E-deficient mice. *Proc Natl Acad Sci USA*. 1996;93:10022–10027.
78. Hodgins JB, Kregel JH, Reddick RL, Korach KS, Smithies O, Maeda N. Estrogen receptor alpha is a major mediator of 17beta-estradiol's atheroprotective effects on lesion size in Apoe^{-/-} mice. *J Clin Invest*. 2001;107:333–340. doi: 10.1172/JCI11320.
79. Billon-Galés A, Fontaine C, Douin-Echinard V, Delpy L, Berges H, Calippe B, Lenfant F, Laurell H, Guéry JC, Gourdy P, Arnal JF. Endothelial estrogen receptor-alpha plays a crucial role in the atheroprotective action of 17beta-estradiol in low-density lipoprotein receptor-deficient mice. *Circulation*. 2009;120:2567–2576. doi: 10.1161/CIRCULATIONAHA.109.898445.
80. Villablanca AC, Tenwolde A, Lee M, Huck M, Mumenthaler S, Rutledge JC. 17beta-estradiol prevents early-stage atherosclerosis in estrogen receptor-alpha deficient female mice. *J Cardiovasc Transl Res*. 2009;2:289–299. doi: 10.1007/s12265-009-9103-z.
81. Arnal JF, Fontaine C, Billon-Galés A, Favre J, Laurell H, Lenfant F, Gourdy P. Estrogen receptors and endothelium. *Arterioscler Thromb Vasc Biol*. 2010;30:1506–1512. doi: 10.1161/ATVBAHA.109.191221.
82. Chow RW, Handelsman DJ, Ng MK. Minireview: rapid actions of sex steroids in the endothelium. *Endocrinology*. 2010;151:2411–2422. doi: 10.1210/en.2009-1456.
83. Mikkola T, Turunen P, Avela K, Orpana A, Viinikka L, Ylikorkala O. 17 beta-estradiol stimulates prostacyclin, but not endothelin-1, production in human vascular endothelial cells. *J Clin Endocrinol Metab*. 1995;80:1832–1836. doi: 10.1210/jcem.80.6.7775630.
84. Mikkola T, Ranta V, Orpana A, Ylikorkala O, Viinikka L. Effect of physiological concentrations of estradiol on PGI2 and NO in endothelial cells. *Maturitas*. 1996;25:141–147.
85. Badeau RM, Metsu J, Wähälä K, Tikkanen MJ, Jauhainen M. Human macrophage cholesterol efflux potential is enhanced by HDL-associated 17beta-estradiol fatty acyl esters. *J Steroid Biochem Mol Biol*. 2009;116:44–49. doi: 10.1016/j.jsbmb.2009.04.008.
86. Frazier-Jessen MR, Kovacs EJ. Estrogen modulation of JE/monocyte chemoattractant protein-1 mRNA expression in murine macrophages. *J Immunol*. 1995;154:1838–1845.
87. Deshpande R, Khalili H, Pergolizzi RG, Michael SD, Chang MD. Estradiol down-regulates LPS-induced cytokine production and NFκB activation in murine macrophages. *Am J Reprod Immunol*. 1997;38:46–54.
88. Vegeto E, Ghisletti S, Meda C, Eteri S, Belcredito S, Maggi A. Regulation of the lipopolysaccharide signal transduction pathway by 17beta-estradiol in macrophage cells. *J Steroid Biochem Mol Biol*. 2004;91:59–66. doi: 10.1016/j.jsbmb.2004.02.004.
89. Dai-Do D, Espinosa E, Liu G, Rabelink TJ, Julmy F, Yang Z, Mahler F, Lüscher TF. 17 beta-estradiol inhibits proliferation and migration of human vascular smooth muscle cells: similar effects in cells from postmenopausal females and in males. *Cardiovasc Res*. 1996;32:980–985.
90. Suzuki A, Mizuno K, Ino Y, Okada M, Kikkawa F, Mizutani S, Tomoda Y. Effects of 17 beta-estradiol and progesterone on growth-factor-induced proliferation and migration in human female aortic smooth muscle cells *in vitro*. *Cardiovasc Res*. 1996;32:516–523.
91. Nguyen CP, Hirsch MS, Moeny D, Kaul S, Mohamoud M, Joffe HV. Testosterone and “age-related hypogonadism”—FDA concerns. *N Engl J Med*. 2015;373:689–691. doi: 10.1056/NEJMp1506632.
92. Casquero AC, Berti JA, Salerno AG, Bighetti EJ, Cazita PM, Ketelhuth DF, Gidlund M, Oliveira HC. Atherosclerosis is enhanced by testosterone deficiency and attenuated by CETP expression in transgenic mice. *J Lipid Res*. 2006;47:1526–1534. doi: 10.1194/jlr.M600135-JLR200.
93. von DG, von DO, Volker W, Langer C, Weinbauer GF, Behre HM, Nieschlag E, Assmann G, von EA. Atherosclerosis in apolipoprotein E-deficient mice is decreased by the suppression of endogenous sex hormones. *Horm Metab Res*. 2001;33:110–114.
94. Henriques TA, Huang J, D'Souza SS, Daugherty A, Cassis LA. Orchiectomy, but not ovariectomy, regulates angiotensin II-induced vascular diseases in apolipoprotein E-deficient mice. *Endocrinology*. 2004;145:3866–3872. doi: 10.1210/en.2003-1615.
95. Mukherjee TK, Dinh H, Chaudhuri G, Nathan L. Testosterone attenuates expression of vascular cell adhesion molecule-1 by conversion to estradiol by aromatase in endothelial cells: implications in atherosclerosis. *Proc Natl Acad Sci USA*. 2002;99:4055–4060. doi: 10.1073/pnas.052703199.
96. Qiu Y, Yanase T, Hu H, Tanaka T, Nishi Y, Liu M, Sueishi K, Sawamura T, Nawata H. Dihydrotestosterone suppresses foam cell formation and attenuates atherosclerosis development. *Endocrinology*. 2010;151:3307–3316. doi: 10.1210/en.2009-1268.
97. McRobb L, Handelsman DJ, Heather AK. Androgen-induced progression of arterial calcification in apolipoprotein E-null mice is uncoupled from plaque growth and lipid levels. *Endocrinology*. 2009;150:841–848. doi: 10.1210/en.2008-0760.
98. Higgins CD, Swerdlow AJ, Schoemaker MJ, Wright AF, Jacobs PA; UK Clinical Cytogenetics Group. Mortality and cancer incidence in males with Y polysomy in Britain: a cohort study. *Hum Genet*. 2007;121:691–696. doi: 10.1007/s00439-007-0365-8.
99. Charchar FJ, Bloomer LD, Barnes TA, et al. Inheritance of coronary artery disease in men: an analysis of the role of the Y chromosome. *Lancet*. 2012;379:915–922. doi: 10.1016/S0140-6736(11)61453-0.
100. Van PL, Bakalov VK, Bondy CA. Monosomy for the X-chromosome is associated with an atherogenic lipid profile. *J Clin Endocrinol Metab*. 2006;91:2867–2870. doi: 10.1210/jc.2006-0503.
101. Gravholt CH, Juul S, Naeraa RW, Hansen J. Morbidity in Turner syndrome. *J Clin Epidemiol*. 1998;51:147–158.
102. Hannawa KK, Eliason JL, Upchurch GR Jr. Gender differences in abdominal aortic aneurysms. *Vascular*. 2009;17(suppl 1):S30–S39.
103. Makrygiannis G, Courtois A, Drion P, Defraigne JO, Kuivaniemi H, Sakalihan N. Sex differences in abdominal aortic aneurysm: the role of sex hormones. *Ann Vasc Surg*. 2014;28:1946–1958. doi: 10.1016/j.avsg.2014.07.008.
104. Cosford PA, Leng GC. Screening for abdominal aortic aneurysm. *Cochrane Database Syst Rev*. 2007;CD002945.
105. Guirguis-Blake JM, Beil TL, Senger CA, Whitlock EP. Ultrasonography screening for abdominal aortic aneurysms: a systematic evidence review for the U.S. Preventive Services Task Force. *Ann Intern Med*. 2014;160:321–329. doi: 10.7326/M13-1844.
106. Grootenboer N, Bosch JL, Hendriks JM, van Sambeek MR. Epidemiology, aetiology, risk of rupture and treatment of abdominal aortic aneurysms: does sex matter? *Eur J Vasc Endovasc Surg*. 2009;38:278–284. doi: 10.1016/j.ejvs.2009.05.004.
107. Skibba AA, Evans JR, Hopkins SP, Yoon HR, Katras T, Kalbfleisch JH, Rush DS. Reconsidering gender relative to risk of rupture in the contemporary management of abdominal aortic aneurysms. *J Vasc Surg*. 2015;62:1429–1436. doi: 10.1016/j.jvs.2015.07.079.
108. Sweeting MJ, Thompson SG, Brown LC, Powell JT; RESCAN collaborators. Meta-analysis of individual patient data to examine factors affecting growth and rupture of small abdominal aortic aneurysms. *Br J Surg*. 2012;99:655–665. doi: 10.1002/bjs.8707.
109. Stuntz M. Modeling the burden of abdominal aortic aneurysm in the USA in 2013. *Cardiology*. 2016;135:127–131.
110. Laser A, Ghosh A, Roelofs K, Sadiq O, McEvoy B, DiMusto P, Eliason J, Upchurch GR Jr. Increased estrogen receptor alpha in experimental aortic aneurysms in females compared with males. *J Surg Res*. 2014;186:467–474. doi: 10.1016/j.jss.2013.07.050.
111. Johnston WF, Salmon M, Su G, Lu G, Ailawadi G, Upchurch GR Jr. Aromatase is required for female abdominal aortic aneurysm protection. *J Vasc Surg*. 2015;61:1565–74.e1. doi: 10.1016/j.jvs.2014.01.032.
112. Martin-McNulty B, Tham DM, da Cunha V, Ho JJ, Wilson DW, Rutledge JC, Deng GG, Vergona R, Sullivan ME, Wang YX. 17 Beta-estradiol attenuates development of angiotensin II-induced aortic abdominal aneurysm in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*. 2003;23:1627–1632. doi: 10.1161/01.ATV.0000085842.20866.6A.
113. Thatcher SE, Zhang X, Woody S, Wang Y, Alsiraj Y, Charnigo R, Daugherty A, Cassis LA. Exogenous 17-β estradiol administration blunts progression of established angiotensin II-induced abdominal aortic aneurysms in female ovariectomized mice. *Biol Sex Differ*. 2015;6:12. doi: 10.1186/s13293-015-0030-1.
114. Henriques T, Zhang X, Yiannikouris FB, Daugherty A, Cassis LA. Androgen increases AT1a receptor expression in abdominal aortas to promote angiotensin II-induced AAAs in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*. 2008;28:1251–1256. doi: 10.1161/ATVBAHA.107.160382.

115. Zhang X, Thatcher SE, Rateri DL, Bruemmer D, Charnigo R, Daugherty A, Cassis LA. Transient exposure of neonatal female mice to testosterone abrogates the sexual dimorphism of abdominal aortic aneurysms. *Circ Res*. 2012;110:e73–e85. doi: 10.1161/CIRCRESAHA.111.253880.
116. Zhang X, Thatcher S, Wu C, Daugherty A, Cassis LA. Castration of male mice prevents the progression of established angiotensin II-induced abdominal aortic aneurysms. *J Vasc Surg*. 2015;61:767–776. doi: 10.1016/j.jvs.2013.11.004.
117. Alsiraj Y, Thatcher SE, Charnigo R, Chen K, Blalock E, Daugherty A, Cassis LA. Female mice with an XY sex chromosome complement develop severe angiotensin II-induced abdominal aortic aneurysms. *Circulation*. 2017;135:379–391. doi: 10.1161/CIRCULATIONAHA.116.023789.
118. Hayward CS, Kelly RP, Collins P. The roles of gender, the menopause and hormone replacement on cardiovascular function. *Cardiovasc Res*. 2000;46:28–49.
119. Parker WH, Jacoby V, Shoupe D, Rocca W. Effect of bilateral oophorectomy on women's long-term health. *Womens Health (Lond)*. 2009;5:565–576. doi: 10.2217/whe.09.42.
120. Song X, Li G, Vaage J, Valen G. Effects of sex, gonadectomy, and oestrogen substitution on ischaemic preconditioning and ischaemia-reperfusion injury in mice. *Acta Physiol Scand*. 2003;177:459–466. doi: 10.1046/j.1365-201X.2003.01068.x.
121. Ostadal B, Netuka I, Maly J, Besik J, Ostadalova I. Gender differences in cardiac ischemic injury and protection—experimental aspects. *Exp Biol Med (Maywood)*. 2009;234:1011–1019. doi: 10.3181/0812-MR-362.
122. Lin J, Steenbergen C, Murphy E, Sun J. Estrogen receptor-beta activation results in S-nitrosylation of proteins involved in cardioprotection. *Circulation*. 2009;120:245–254. doi: 10.1161/CIRCULATIONAHA.109.868729.
123. Lagranha CJ, Deschamps A, Aponte A, Steenbergen C, Murphy E. Sex differences in the phosphorylation of mitochondrial proteins result in reduced production of reactive oxygen species and cardioprotection in females. *Circ Res*. 2010;106:1681–1691. doi: 10.1161/CIRCRESAHA.109.213645.
124. Albrektsen G, Heuch I, Løchen ML, Thelle DS, Wilsgaard T, Njølstad I, Børnaa KH. Lifelong gender gap in risk of incident myocardial infarction: the Tromsø study. *JAMA Intern Med*. 2016;176:1673–1679. doi: 10.1001/jamainternmed.2016.5451.
125. Mosca L, Barrett-Connor E, Wenger NK. Sex/gender differences in cardiovascular disease prevention: what a difference a decade makes. *Circulation*. 2011;124:2145–2154. doi: 10.1161/CIRCULATIONAHA.110.968792.
126. Parker WH. Ovarian conservation versus bilateral oophorectomy at the time of hysterectomy for benign disease. *Menopause*. 2014;21:192–194. doi: 10.1097/GME.0b013e31829be0a0.
127. World Heart Federation. <http://www.world-heart-federation.org/cardiovascular-health/cardiovascular-disease-risk-factors/hypertension/>.
128. Sandberg K, Ji H. Sex differences in primary hypertension. *Biol Sex Differ*. 2012;3:7. doi: 10.1186/2042-6410-3-7.
129. Pollock DP, Uhrlaub J, Romero-Aleshire MJ, Sandberg K, Nikolich-Zugich J, Brooks HL, Hay M. Sex differences in T-lymphocyte tissue infiltration and development of angiotensin II hypertension. *Hypertension*. 2014;64:384–390. doi: 10.1161/HYPERTENSIONAHA.114.03581.
130. Ji H, Zheng W, Li X, Liu J, Wu X, Zhang MA, Umans JG, Hay M, Speth RC, Dunn SE, Sandberg K. Sex-specific T-cell regulation of angiotensin II-dependent hypertension. *Hypertension*. 2014;64:573–582. doi: 10.1161/HYPERTENSIONAHA.114.03663.
131. Ji H, Zheng W, Wu X, Liu J, Ecelbarger CM, Watkins R, Arnold AP, Sandberg K. Sex chromosome effects unmasked in angiotensin II-induced hypertension. *Hypertension*. 2010;55:1275–1282. doi: 10.1161/HYPERTENSIONAHA.109.144949.
132. Pessôa BS, Slump DE, Ibrahim K, Grefhorst A, van Veghel R, Garrelts IM, Roks AJ, Kushner SA, Danser AH, van Esch JH. Angiotensin II type 2 receptor- and acetylcholine-mediated relaxation: essential contribution of female sex hormones and chromosomes. *Hypertension*. 2015;66:396–402. doi: 10.1161/HYPERTENSIONAHA.115.05303.
133. Vasan RS, Larson MG, Leip EP, Evans JC, O'Donnell CJ, Kannel WB, Levy D. Impact of high-normal blood pressure on the risk of cardiovascular disease. *N Engl J Med*. 2001;345:1291–1297. doi: 10.1056/NEJMoa003417.
134. D'Agostino RB Sr, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, Kannel WB. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation*. 2008;117:743–753. doi: 10.1161/CIRCULATIONAHA.107.699579.
135. Dantas AP, Franco Mdo C, Silva-Antonialli MM, Tostes RC, Fortes ZB, Nigro D, Carvalho MH. Gender differences in superoxide generation in microvessels of hypertensive rats: role of NAD(P)H-oxidase. *Cardiovasc Res*. 2004;61:22–29.
136. Arnold AP, Chen X, Link JC, Itoh Y, Reue K. Cell-autonomous sex determination outside of the gonad. *Dev Dyn*. 2013;242:371–379. doi: 10.1002/dvdy.23936.
137. Murphy E, Steenbergen C. Sex, drugs, and trial design: sex influences the heart and drug responses. *J Clin Invest*. 2014;124:2375–2377. doi: 10.1172/JCI76262.
138. *Incidence and Prevalence: 2006 Chart Book on Cardiovascular and Lung Diseases*. Bethesda MD: National Heart, Lung, and Blood Institute; 2016.

Highlights

- Study of sex differences in cardiovascular physiology and disease in mice allows one to break down biological sex into its component parts, so that harmful or protective effects of hormones and sex chromosome genes can be identified.
- In mice, estrogens are generally protective in various models of cardiovascular and associated diseases.
- XX and XY animals differ in cardiovascular physiology and disease, independent of the type of gonadal hormones.
- The biological factors protecting one sex from disease, more than the other, may potentially be targeted in novel therapies.

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