Sex hormones in the cardiovascular system

Abstract: Gender-associated differences in the development of cardiovascular diseases have been described in humans and animals. These differences could explain the low incidence of cardiovascular disease in women in the reproductive period, such as stroke, hypertension, and atherosclerosis. The cardiovascular protection observed in females has been attributed to the beneficial effects of estrogen on endothelial function. Besides estrogen, sex hormones are able to modulate blood pressure by acting on important systems as cardiovascular, renal, and neural. They can have complementary or antagonistic actions. For example, testosterone can raise blood pressure by stimulating the renin-angiotensin-aldosterone system, whereas estrogen alone or combined with progesterone has been associated with decreased blood pressure. The effects of testosterone in the development of cardiovascular disease are contradictory. Although some researchers suggest a positive effect, others indicate negative actions of testosterone. Estrogens physiologically stimulate the release of endothelium-derived vasodilator factors and inhibit the renin-angiotensin system. Although the cardioprotective effects of estrogen are widely appreciated, little is known about the effects of progesterone, which is commonly used in hormone replacement therapy. Progesterone has both vasodilatory and vasoconstrictive effects in the vasculature, depending on the location of the vessel and the level of exposure. Nevertheless, the mechanisms through which sex hormones modulate blood pressure have not been fully elucidated. Therefore, the characterization of those could lead to a better understanding of hypertension in women and men and perhaps to improved forms of therapy.

Keywords: cardioprotection; sex hormones; vascular and cardiac function.

List of abbreviations

ACE Angiotensin converting enzyme
AR Androgen receptor
DHT Dihydrotestosterone
ER Estrogen receptor
ERRs Estrogen related receptors
GPER G protein–coupled estrogen receptor
HERS Heart and Estrogen/Progesterone Replacement Study
HRT Hormone replacement therapy
HT Hormone therapy
mPR Membrane receptors for progesterone
NHANES National Health and Nutrition Examination Survey
OSH Ovarian sex hormones
PLB Phospholamban
PR Progesterone receptor
RUTH Raloxifene Use for the Heart
SERCA2a Sarcoplasmic reticulum calcium pump
SERMs Selective estrogen receptor modulators
WHI Women’s Health Initiative

Introduction

Cardiovascular diseases are major contributors to morbidity and mortality among postmenopausal women in the Western world [1]. Interestingly, premenopausal women have a reduced risk of mortality from cardiovascular disease, whereas postmenopausal women have a similar or even increased risk for cardiovascular disease as compared with men [2]. It has been suggested that ovarian sex hormones (OSH) have a protective effect on the cardiovascular system [2, 3]. However, the effects of estrogen replacement therapy on the cardiovascular risk only account for about 50% of the reduction seen in cardiovascular disease, suggesting that there must be additional mechanisms whereby estrogen exerts its cardioprotective effects [4].

Sexual dimorphism is apparent in humans as well as most, if not all, mammals. Numerous studies have shown that human females exhibit lower levels over much of their life span compared with their age-matched...
male counterparts. Steroid hormones have roles in the regulation of a wide variety of bodily processes including regulation of blood pressure. As such, they appear to be important factors in the development of hypertension and coronary artery disease, some of the most common cardiovascular diseases in the industrialized world [5, 6]. For example, after the onset of menopause blood pressure levels increase and become similar to those in men, suggesting an important role of sex hormones in the regulation of blood pressure [7, 8]. These data provide evidence that sex hormones influence the cardiovascular system; however, these influences are still poorly understood.

There are observations that suggest both estrogen deficiency in women and androgen deficiency in man might contribute to the development of cardiovascular disease and hypertension [9, 10]. Most epidemiological studies have found that there is a high prevalence of low testosterone levels in men with coronary heart disease, and that this association exists regardless of the age of the patient [9]. Additionally, considerable evidence now suggests that testosterone and other androgens have protective effects on cardiovascular and may play important roles in the acute regulation of vascular function [10]. Indeed, studies have demonstrated that testosterone exerts beneficial effects on cardiovascular function by inducing rapid vasorelaxation of vascular smooth muscle [11].

Although the exact mechanisms by which sex hormones contribute to the regulation of cardiovascular function and blood pressure are still being investigated, there is increasing evidence that modulating the activity of locally active hormone systems is one of the major mechanisms of sex hormone actions in target organs, including the vasculature and kidneys [8, 12]. Estrogen, progesterone, and testosterone receptors have been identified in blood vessels where they appear to cause a stimulation of endothelium-dependent mechanisms of vascular relaxation and inhibition of the mechanisms of vascular smooth muscle contraction. These potential actions might contribute to the gender differences in vascular tone and represent potential beneficial vascular effects of hormone replacement therapy (HRT) during natural and surgically induced deficiencies of gonadal hormones [5, 12, 13].

**Mechanism of action of sex hormones**

The estrogen receptor (ER) exists in three main forms, ERα, ERβ, and G-protein-coupled estrogen receptor (GPER) (Figure 1), the latter being also known as G-protein-coupled receptor 30 (GPR30) and as the membrane ER [14, 15]. These receptors are present in both genders and are widely distributed in many organs, including cardiovascular and reproductive systems, liver, brain, and bone. ERα and ERβ are found both in associations with the plasma membrane, in the cytoplasm and in the nucleus [16]. Some of the known functions of ERα are related to the activation of eNOS [17, 18], prevention of vascular smooth muscle cells (VSMCs) proliferation [19], and inhibition of increase in medial thickness [20], whereas ERβ reduces pathological cardiac hypertrophy [21], inhibit cardiac fibrosis [22], and is related to the development of sustained systolic and diastolic hypertension in ERβ-deficient mice as they age [14]. Both ERs are necessary and sufficient for estrogen-mediated protection against measures of vascular injury in mice [14] and may activate phosphoinositide 3-kinase (PI3K), AKT, and mitogen-activated protein kinase (MAPK) pathways [16]. The GPER was cloned in 1997 from shear-stress-exposed human endothelial cells [23]. It can induce relaxation in porcine aortic rings and human coronary artery, which is dependent on the large-conductance calcium (BKCa) and voltage-activated potassium channels (Kv) activation [24]. Additionally, GPER can activate the nonnuclear protective response, i.e., PI3-kinase, Akt, via a truncated 36-kDa ERα [25] and attenuate diastolic dysfunction and ventricular remodeling in ovariecetomized rats. Endogenous human 17β-estradiol, selective estrogen receptor modulators (SERMs) including tamoxifen and raloxifene, and selective estrogen receptor downregulators (SERDs) such as ICI 182,780 are all agonists of GPER, which has been implicated in the regulation of vasomotor tone and protection from myocardial ischemia/reperfusion injury. As a result, understanding the individual roles of ERα, ERβ, and GPER in cardiovascular function has become increasingly complex [26]. Some estrogen’s actions can be evoked by estrogen binding to other receptors, such as estrogen-related receptors (ERRs). These receptors are orphan nuclear receptors, with three isoforms – β and γ. The ERRs are involved in energy metabolism and mitochondrial biogenesis, and their activity depends on coactivator proteins [27–29]. However, little is known about the interaction between estrogen and ERRs and other possible ligands for these receptors.

Estrogen diffuses through the plasma membrane and forms complexes with cytosolic and nuclear ER (Figure 1), which bind to chromatin, stimulate gene transcription, and induce genomic effects. For example, the estrogen deficiency in young adult rats resulted in increased expression of a number of pro-inflammatory genes in the heart including L-selectin, calpain, tumor necrosis factor, iNOS, and fibronectin [30].
The physiological consequence of non-genomic effects, in most cases, is a rapid vasodilatation caused by activating endothelial factor production, opening of potassium channels [31], and activation of numerous signal transduction cascades that support the favorable effects of estrogen on vascular structure and function [32]. Estrogen binds to signal-generating ER on the plasma membrane of vascular cells and induces rapid non-genomic endothelium-dependent and -independent signaling cascades [PI3K and Akt as well as ERK, extracellular signal-regulated kinases (ERK1/2), c-Jun NH2-terminal kinase (JNK), and p38] [33, 34] and vascular effects [5]. Rapid vasodilatory effects of estrogen are produced by estrogen-stimulated increases of eNOS activity [35, 36]. Caulin-Glaser et al. [37] demonstrated a rapid increase in nitric oxide (NO) from female umbilical vein endothelial cells in response to 17\(\beta\)-estradiol. Chen et al. [38] showed that ER\(\alpha\) can mediate the short-term effects of estrogen on eNOS activity within 5 min. Additionally, Chambliss et al. [39] demonstrated that ER\(\beta\) mediates non-genomic effects on eNOS in plasma membrane caveolae. GPER has also been shown to bind estradiol and induce rapid signaling events [40]. The molecular mechanisms by which estrogen causes a rapid vasodilation is an area of intense current interest, and the contributions by the various pathways need to be further defined.

Similar to estrogen, progesterone effects are mediated by binding to the nuclear progesterone receptor (PR) [41]. Receptors for progesterone are expressed as two distinct isoforms, PR-A and PR-B (Figure 1) that arise from a single gene [42]. These isoforms are present in various human tissues including the uterus, mammary gland, brain, pancreas, bone, ovary, testes, and tissues of the lower urinary tract. The ubiquitous expression of PR highlights the widespread physiological effects that progesterone can exert in a variety of organs throughout the body [41]. The general pathway of progesterone inducible PR-mediated gene transcription has been well characterized. Progesterone binding induces a conformational change in PR that promote dissociation from a multi-protein chaperone complex, homodimerization, and binding to specific progesterone response elements within the promoter of target

![Figure 1](image-url)
genes. DNA bound receptors increase or decrease rates of gene transcription by influencing recruitment of RNA polymerase II to the initiation site. Through protein-protein interaction, hormone-activated PR recruits coactivators that serve as essential intermediates for transmitting signals from the receptor to the transcription initiation complex. Coactivators facilitate transcription initiation through protein interactions with components of the general transcription machinery and by promoting local remodeling of chromatin at specific promoters [43].

A new class of PRs located in the plasma membrane was found initially in spotted sea trout ovaries [44] and subsequently detected in human tissues [45]. Membrane receptors for progesterone (mPR) has three isoforms (mPRα, mPRβ, and mPRγ) that are structurally unrelated to nuclear steroid receptors (Figure 1), but instead has features typical of G-protein-coupled receptors. The non-genomic mechanisms of action of the mPRs are typically coupled to the inhibitory G-protein Gαi, whereby progesterone stimulation decreases 3',5'-cyclic adenosine monophosphate (cAMP) synthesis, which is sensitive to pertussis toxin inhibition [46]. mPR stimulation also causes activating phosphorylations of the MAPKs ERK1/2 and JNK1/2 [45].

All three isoforms of mPRs have been shown to bind progesterone with high affinity, but not with either synthetic progestins or anti-progestins [47]. The androgen receptor (AR) exists in two forms, ARα and ARβ, which have distinct tissue expression patterns [48]. Sex differences in the distribution and abundance of AR have been demonstrated in a variety of tissues including VSMCs and endothelial cells [49]. Testosterone also binds to an intracellular receptor (Figure 1), and this receptor-steroid complex then binds to DNA in the nucleus, facilitating transcription of various genes. In some target cells, testosterone is converted to dihydrotestosterone (DHT) by 5α-reductase and DHT binds to the same intracellular receptor as testosterone. Testosterone-receptor complexes are less stable than DHT-receptor complexes in target cells, and they conform less well to the DNA-binding state. Thus, DHT formation is a way of amplifying the action of testosterone in target tissues. In addition to genomic effect, testosterone can induce non-genomic effect by membrane receptors. The non-genomic effects are rapidly produced and do not require the association of AR to DNA. These effects involve the activation of various signaling pathways, including calcium, protein kinase A, protein kinase C, and MAPK [50].

Thus, both the male and female sex hormones are able to act through genomic and non-genomic mechanisms. These hormones seem to modulate blood pressure [51]. However, although the activation of ER is associated with the reduction or attenuation of blood pressure, the action of male sex hormones leads to an elevation in blood pressure.

Sex hormones in the cardiovascular system

Sex hormones have a role in the regulation a wide range of physiological processes, including blood pressure. Thus, they might be important factors in the development of hypertension and coronary artery disease, some of the most common cardiovascular diseases in the industrialized world [12]. For example, the high levels of endogenous estrogens in premenopausal women have been associated with lower risk for a number of diseases, such as hypertension, diabetes mellitus, obesity, vascular disease, and stroke compared with age-matched men [6, 15]. In addition, data from the National Health and Nutrition Examination Survey indicate that age-standardized hypertension prevalence rates are increasing in general, and there is no subgroup in which they are declining, and among each race/ethnicity, these trends are more favorable in men than in women. However, this clear female benefit no longer exists after menopause, showing again the influence of sex hormones in the pathophysiology of cardiovascular disease. Nevertheless, the impact of testosterone on the cardiovascular system is controversial. Elderly men are typically at higher risk for adverse cardiovascular events than age-matched women, and one study suggested that exogenous testosterone was associated with an increase in adverse cardiovascular events in this population [52]. In contrast, other clinical studies suggest that testosterone is beneficial to the cardiovascular system and that low levels of testosterone negatively affect the cardiovascular system [53, 54]. Indeed, there is no compelling evidence that testosterone replacement to levels within the normal healthy range contributes adversely to the pathogenesis of cardiovascular disease [55]. Obviously, the use of testosterone therapy that is beneficial, or at least safe, should be based both on thorough understanding of relationship between endogenous androgens and cardiovascular disease in conditions of health and disease and on findings of controlled trials with relevant end-points, with appropriate design, and of sufficient duration. Unfortunately, experimental evidence for potential beneficial or adverse effects of testosterone on the cardiovascular system is rather limited. There are no published reports of randomized clinical trials with the primary goal to evaluate the effects...
of testosterone on the incidence of cardiovascular events. Many of the trials to evaluate the effects of testosterone were conducted in disease men [56–58], thereby limiting interpretation and generalization of results. In the following sections, we will discuss the cardiac and vascular functions of sex hormones in detail.

**Sex hormones and cardiac function**

Several studies have also indicated that estrogens might have cardioprotective actions on myocardial contractility [59, 60]. Estrogens alters the myocardial expression of the following mediators of cardiac contractility: ventricular β1-adrenoreceptor [61, 62], intracellular calcium homeostasis related to the L-type Ca$^{2+}$ channel (LTCC) [63], cardiac sarcoplasmic reticulum Ca$^{2+}$ uptake [64], and Ca$^{2+}$ sensitivity of cardiac myofilaments in ovariectomized rats [65]. Although a cardiac transcriptional regulation of estrogen is well described, there is little direct evidence of membrane cardiac ERs. Data on the non-genomic signal transduction in cardiomyocytes have demonstrated that 17β-estradiol has a negative inotropic effect on guinea pig single ventricular myocytes produced by inhibiting ICa$^{2+}$ and thus reducing systolic Ca$^{2+}$ [66]. Furthermore, in isolated rat ventricular cardiomyocytes, it was demonstrated that estrogens exerts opposite effects on the intracellular pH of myocytes, which is associated with both pro- and anti-hypertrophic effects [67]. One important question is whether OSH affect the myocardium directly or by secondary mechanisms involving other hormones modulated by estrogens.

Studies have indicated that OSH therapy restores contractile performance in postmenopausal women [68–70]. Recent studies further suggest that mechanical functioning and proteomic profiles in ventricular myocytes are directly regulated by estrogens [61, 64, 71, 72]. The long-term deficiency of OSH reduces cardiac contractility associated with changes in the expression of key contractile proteins, sarcoplasmic reticulum calcium pump (SERCA2a), and phospholamban (PLB) [61, 64, 71–73]. Paigel et al. [73] demonstrated that in rats at 60 days after ovariectomy, the PLB/SERCA2a ratio was increased by approximately 1.6-fold and it was restored to control values after 17β-estradiol treatment. No changes in the expression of sodium-calcium exchanger protein were observed following ovariectomy. The results of transgenic and gene-targeted studies in mice suggested that changes in the PLB/SERCA2a ratio might be a major factor in altering cardiac contractility [74, 75]. In vivo studies using a strain of transgenic mice that over expresses cardiac specific PLB suggested that the “functional stoichiometry” of PLB/SERCA2 is >1:1 in native cardiac sarcoplasmic reticulum membranes [75]. In these transgenic mice, there was a 2-fold higher level of PLB as compared with non-transgenic mice that resulted in a greater inhibition of Ca$^{2+}$-ATPase affinity for Ca$^{2+}$, which was associated with decreases in contractility and Ca$^{2+}$ transport in to cardiomyocytes. Because 17β-estradiol replacement restored sarcoplasmic reticulum protein expression and myocardial contractility, it is plausible that OSH participate in the long-term regulation of cardiac contractility. In fact, a previous study observed thyroid hormone regulation of sarcoplasmic reticulum protein expression and cardiac contractility [76]. These authors also observed enhanced cardiac PLB expression that was associated with decreased rates of cardiac sarcoplasmic reticulum Ca$^{2+}$ uptake, which is consistent with increased inhibition of SERCA2a and decreased contractility in hypothyroid rats.

The results of long-term studies demonstrated the influence of OSH on rat cardiac contractility [69] through impaired left ventricular function. Impaired function was characterized by decreases in cardiac output, peak systolic pressure, and ejection fraction at all preloads in the ovariectomized rat hearts before puberty. These contractile changes were further associated with a decrease in myosin ATPase activity. Another study [70] demonstrated that the same changes in cardiac function were reversed by 17β-estradiol replacement. Changes in cardiomyocyte intracellular Ca$^{2+}$ homeostasis suggested a possible myocardial dysfunction resulting from a deficiency of OSH [64, 65]. The regulatory role of OSH in the calcium uptake activity of cardiac sarcoplasmic reticulum was also demonstrated in 10-week ovariectomized rat [64]. These authors demonstrated that estrogen and progesterone supplementation were equally effective in preventing changes in hearts from ovariectomized animals.

Proudler et al. [77] demonstrated that estrogen replacement therapy is able to decrease angiotensin-converting enzyme (ACE) activity and the risk of coronary artery disease in women. Results of animal studies also showed that ACE activity and levels of type 1 angiotensin II receptor (AT$_{1}$) are reduced by estrogen therapy [78]. According to Gallagher et al. [79], the reduction in ACE activity was not due to a direct interaction of estrogen with the enzyme; rather, it seems that estrogen regulates ACE mRNA synthesis at the tissue level. Thus, estrogen replacement therapy may contribute to enhanced cardiovascular protection by down regulating ACE, thereby reducing angiotensin II levels. Angiotensin II is an important factor in cardiac remodeling and left ventricular dysfunction, and tissue angiotensin II is increased in failing hearts [80, 81]. In fact, Ribeiro et al. [82] demonstrated that daily
administration of losartan to block AT₁ receptors initiated immediately following ovariectomy prevented cardiac contractile dysfunction and oxidative stress observed at 58 days after ovariectomy. This response was associated with changes in expression levels of two key proteins involved in calcium homeostasis, SERCA2a and PLB. Losartan treatment reversed the ovariectomy-induced contractile dysfunction, restored the SERCA2a and PLB levels to control values, and also diminished reactive oxygen species formation. Furthermore, AT₁ receptor blockade attenuated the increase in p22phox expression but did not affect the increased ACE activity in the ovariectomized group. It has been shown that testosterone also has effects on the cardiac muscle. It should be noted that cardiac muscle has AR [83]. In experimental myocardial infarction in rats and mice, testosterone treatment caused maladaptive myocardial hypertrophy, with an increase in the β/α-MHC ratio and in IGF-1 expression [84, 85]. Meanwhile, Chen et al. [86] showed that testosterone insufficiency impaired the mobilization and homing of CD34+ stem cells and expression of hypoxia-inducible factor 1a, stromal cell-derived factor 1a, and vascular endothelium growth factor in ischemic myocardium in the early stage of myocardial infarction. Chen et al. [86] also found that testosterone replacement therapy reversed these changes, and resulted in a concomitant increase in neovascularization. In addition, Kang et al. [87] demonstrated that the castration increased type 2 angiotensin II receptor (AT₂) expression, cardiac fibrosis, and cardiac cell apoptosis in rats with heart failure, which were reduced following testosterone replacement.

Regarding the electrical activity of the heart, both acute intravenous and chronic transdermal testosterone therapy delay the onset of exercise-induced S-T segment depression in men with chronic, stable angina [56, 88]. The steroid also extends total exercise time before development of myocardial ischemia and shortens return of the S-T segment to the isoelectric line [88].

**Sex hormones and vascular function**

Numerous vascular effects of sex hormones including modulation of vascular function, inflammatory response, metabolic, and hemodynamic effects are attributed to female sex hormones. The vascular protection conferred by estrogen may be mediated indirectly by its influence on the metabolism of lipoproteins [89] and extraneuronal uptake of noradrenalin [90] or by a direct action on the modulation of molecular pathways in the vessel wall and more specifically on endothelial cells [91].

Many studies have disclosed that arterial relaxation mediated by estrogen could result from endothelial cell activation, which releases NO and leads to 3′,5′-cyclic guanosine monophosphate (cGMP) pathway activation in VSMC, which could activate calcium-activated potassium channels. However, this pathway is probably not the main route through which estrogen elicits vasorelaxation because it has been demonstrated several times that estrogen can relax endothelium denuded arteries [92]. Vascular relaxation induced by estrogen in vascular smooth muscle was correlated with decreased Ca²⁺ influx [93, 94] and increased K⁺ efflux [95, 96].

The hormone 17β-estradiol is a potent stimulus for eNOS activation and NO release [92]. NO is a potent vasodilator and inhibitor of platelet aggregation and adhesion and proliferation of VSMCs. In addition, it prevents the development of atherosclerosis. Estrogen also has a modulating effect on constrictive factors and positively upregulates the production of endothelium-derived relaxing factors such as prostacyclin (PGI₂) and the endothelium-derived hyperpolarizing factors (EDHFs) [31], both of which are important mediators of vascular relaxation in resistance arteries (Figure 2). In addition, estrogen increases NO bioavailability by mechanisms that either directly increases NO generation or decrease superoxide anion (O₂⁻) concentration, thereby attenuating O₂⁻-mediated NO inactivation. Estrogens such as 17β-estradiol, estrone, and estriol have been described to act as reactive oxygen species scavengers by virtue of the hydrogen-donating capacity of their phenolic molecular structure.

The peripheral actions of estrogen could reduce the development of hypertension through upregulation of endothelium-derived vasodilator factors with simultaneous downregulation of vasoconstrictor factors such as endothelin 1 [97] and inhibition of the renin-angiotensin system by reducing transcription of ACE [98]. Depletion of estrogen with a concomitant increase in androgens would, therefore, reduce local inhibitory signals while increasing pro-contractile signals at the vascular wall, leading to increased peripheral resistance and blood pressure in the absence of concomitant decreases in sympathetic tone.

Little is known about progesterone effects on vascular bed [99]. Progesterone is a vasoactive hormone that predominantly has vasodilatory actions on several vascular beds, although some studies have reported conflicting effects. Indeed, some studies have shown beneficial effects of progesterone [99, 100], whereas others show negative effects [101]. Similar to estrogen, progesterone has anti-atherogenic effects, decreasing low-density lipoprotein cholesterol (LDL-C) and increasing high-density lipoprotein cholesterol (HDL-C) [102]. Meanwhile,
progesterone antagonizes the antioxidant effects of estrogen and enhances NADPH oxidase activity and production of reactive oxygen species in ovariectomized mice [101]. Nevertheless, most studies have shown the beneficial effects of progesterone on blood vessels. Experimental studies have associated these vascular effects to a dependent interaction progestogen type that binds to receptor. For example, natural progesterone in cultured human umbilical vein endothelial cells has been shown to increase the synthesis of NO, protein expression of eNOS, and activity eNOS, although it was not able to potentiate these effects in the presence of estrogen. In addition, medroxyprogesterone acetate does not change any of these parameters – it inhibited estrogen effects on NO synthesis [103].

The vascular action of testosterone in turn seems to be mediated by both endothelial-dependent and independent mechanisms. These are concentration dependent. The results of in vitro studies show that vasorelaxation induced at the lower end of the concentration response curve of testosterone appears to be partially endothelium dependent and the vasorelaxation induced by higher concentrations of testosterone appears to be largely endothelium independent [104–106]. The differing results of in vitro and in vivo studies on the role and/or the extent of the endothelium in testosterone-induced vasorelaxation appear to be the consequences of differences in individual study designs with respect to the concentration of testosterone used, acute or chronic exposure, the vascular bed investigated, and precontractile agent [107, 108].

Although some studies show a role for the endothelium, testosterone acts predominantly on the vascular smooth muscle. Indeed, a variety of studies have demonstrated that the key mechanism underlying the vasorelaxing action of testosterone is associated with the modulation of VSMC membrane ion channels for Ca^2+ and K^+. These actions include an inactivation of voltage-operated LTCC [109, 110] and an activation of K^+ channels [111, 112] (Figure 2). These ion channel modulations produced by testosterone that results to vasorelaxation involve intracellular signal transduction pathways that
increase the levels of cGMP [113] and cAMP [114]. Deenadayalu et al. [113] showed that testosterone-induced relaxation in endothelium-denuded coronary arteries is mediated, in part, by enhanced NO production that leads to an increase in cGMP synthesis and protein kinase G activation, which in turn opens BKCa channels. Testosterone stimulates NO production by nNOS, which in turn evokes the formation of cGMP (guanylyl cyclase) to induce vasorelaxation [113]. Montaño et al [114] showed that testosterone stimulates cAMP production in single rat aortic myocytes. The possible path leading to vasorelaxation following an increased level of cAMP is an increase in the phosphorylation of protein kinase A [115]. The modulation of intracellular cAMP by testosterone can be caused by the inhibition of cAMP-phosphodiesterase activity, and this inhibition is associated with an increase in intracellular levels of cAMP [116]. Therefore, the underlying cellular and molecular mechanisms by which testosterone modulates vascular reactivity require further elucidation [117].

**New perspectives on the treatment of cardiovascular disease by sex hormones**

The years proximate to the menopause are accompanied by a rise in blood pressure and an increasing prevalence of hypertension [118]. Among the known cardiovascular risk factors, the incidence of both dyslipidemia and hypertension are prevalent. The prevention of cardiovascular disease and its management in postmenopausal women are therefore a major health issue [119].

Hormone therapy (HT) in postmenopausal women has been thought to provide an efficient protection against the progression of arterial disease by correcting the estrogen deficiency [119]. Observational studies show that postmenopausal women who receive HRT have a lower rate of cardiovascular disease and cardiac death than those not receiving HRT [120]. Nevertheless, these studies contrast with the large prospective clinical trials, Heart and Estrogen/Progestin Replacement Study (I and II) and Women's Health Initiative (WHI), where no reduction in cardiovascular events were shown in postmenopausal women on HRT [121]. In fact, these trials suggested that HRT was associated with increased risks in the development of stroke, venous thromboembolic disease, and deep vein thrombosis [122].

The reasons for this paradoxical characterization of HRT have been extensively discussed. Many potential factors might have contributed to the adverse outcome, among them the age of patients, preexisting cardiovascular disease and/or risk, when HRT was initiated, the type of HRT given (conjugated equine estrogen with progestin), dosage, and the thromboembolic properties of estrogen and progestin [119].

In fact, in most observational studies, women started HRT around the time of menopause, which occurs on average at 51 years, whereas in others, the average age of women entering in clinical trials, such as WHI, was 65 years and older. The women in the late-onset studies represent study populations that have some degree of aging associated vascular damage. In addition, participants had been estrogen deficient for an average of 10 years before starting HRT, a relatively late start that could modify the status of ER and molecular signaling so as to attenuate the benefits of estrogen.

Despite the controversy about the benefits of estrogen replacement therapy in postmenopausal women, much recent interest has focused on whether replacing ovarian hormones can change the pattern of blood pressure alterations and affect the natural history of hypertension in postmenopausal women. There is experimental evidence that HRT reduces the incidence of hypertension in postmenopausal women by exerting a direct effect on blood vessels through modulation of endogenous vasoconstrictors and vasodilators [123].

The cardiovascular benefits of estrogen have been attributed to its modulator effect on NO generation by nitric oxide synthase (NOS) isoforms [124]. Estrogen deficiency, induced by ovariectomy, further increased aging-associated vasoconstriction and impaired NO signaling [125]. Estrogen replacement also improved flow-induced vasodilatation in coronary arterioles of aged and ovariectomized rats and restored eNOS phosphorylation (Ser176), suggesting a positive regulation of estrogen on eNOS activity in old females [126]. In addition, untreated postmenopausal women present a reduction in circulating levels of NO increment on endothelin-1 levels and impairment of endothelial function compared with HRT-treated postmenopausal women [123].

Prospective studies in humans have shown variable effects of HRT on blood pressure in normotensive and hypertensive postmenopausal women [123]. The Postmenopausal Estrogen/Progestin Interventions trial found no significant difference in blood pressure as a function of HRT (conjugated equine estrogen with or without native or synthetic progestin) usage. Meanwhile, the WHI found an increase in blood pressure with HRT (conjugated equine estrogen and medroxyprogesterone acetate). Furthermore, several recent clinical trials have uniformly demonstrated that HRT in combination with drospirenone/17β-estradiol reduces blood pressure in hypertensive postmenopausal women. In addition, it has an additive lowering effect on
blood pressure when administered in combination with antihypertensive therapy with ACE inhibitors, angiotensin receptor antagonists, and hydrochlorothiazide [118].

Currently, data concerning the influence of HRT on blood pressure in postmenopausal women are inconclusive because of the limitations of published studies, as discussed previously.

**HRT alternative – SERMs**

SERMs are nonsteroidal molecules with tissue-selective estrogen agonist and antagonist effects. These compounds interact with the ER at the ligand-binding domain and differentially recruit coactivators or corepressors to produce their effects [127]. The SERMs chosen for this study are the most widely prescribed, tamoxifen and raloxifene. Tamoxifen has an estrogen antagonist activity in the breast but an agonist action in the endometrium [128] and is used for the treatment of estrogen-sensitive breast cancer. Raloxifene has antagonist actions in the breast and uterus but agonist actions in the bone [129] and is widely used for the prevention and treatment of osteoporosis [121]. Evidence has shown that raloxifene, like estrogen, induces endothelium-dependent acute vasodilation [130, 131] and directly activates NO synthesis in endothelial cells, resulting in dilation of blood vessels.

It is well known that the physiological effects of estrogen on gene transcription are determined by a characteristic combination of an activator and co-repressor proteins that interact with any given cell. Manipulation of these interactions may provide a rational basis for selectively targeting the cardiovascular system. Raloxifene is approved for the treatment and prevention of osteoporosis in postmenopausal women. Raloxifene has properties similar to estrogen on the cardiovascular system, such as reduction of the concentrations of serum cholesterol and LDL, improvement of endothelial function, and antagonist actions to the proliferative effects of estrogen on the uterus and breast. These properties of raloxifene provided the basis for the Raloxifene Use for the Heart (RUTH) randomized, controlled trial that studied its ability to decrease fatal myocardial infarction, fatal coronary disease, and hospitalization for acute coronary syndrome. The RUTH trial commenced in 2001, with an average 5-year follow-up, and included 10,000 postmenopausal women who were 55 years or older. In RUTH, a reduced risk of invasive breast cancer was observed, but no effects on primary coronary events were seen. Moreover, there was an increased risk of venous thromboembolism and fatal stroke. Thus, further studies are needed to investigate its use in HT.

Tamoxifen is widely used and highly effective as adjuvant therapy for the management of breast cancer patients with ER-positive tumors [132]. Originally developed in the 1960s as a contraceptive agent, tamoxifen was later found to stimulate ovulation as well as inhibit the formation of mammary tumors in rats exposed to carcinogens. Although tamoxifen itself is a poor ER ligand, its metabolite 4-hydroxytamoxifen is a potent antagonist with high affinity for the ER. Tamoxifen and its metabolites exert anti-estrogenic effects in breast tissue, estrogenic effects on bones (increased density) and plasma cholesterol (decreased levels), and mixed effects in the uterus. In addition to the beneficial effects of tamoxifen, its complex, tissue-specific action can also result in adverse effects in women. Perimenopausal symptoms of hot flushes and night sweats are frequently reported side effects in both premenopausal and postmenopausal women on tamoxifen therapy. In premenopausal women, tamoxifen has also been reported to cause oligomenorrhea or amenorrhea with a return to normal menses after discontinuing tamoxifen. Yet the greatest concern has been focused on the effects of tamoxifen in the uterus. Tamoxifen treatment increases the relative risk for endometrial cancer and may increase the incidence of endometriosis and uterine mesenchymal (i.e., myometrial) tumors.

Tamoxifen can also alter vascular reactivity [133] by various pathways including antagonism of several ion channels and calmodulin and inhibition of myosin light chain kinase. These actions are consistent with studies speculating that several potentially important actions of tamoxifen are ER independent [134]. Clinical observations have shown that treatment with tamoxifen reduces the incidence of ischemic heart disease and coronary atherosclerosis. Other studies have also reported decreases in total serum cholesterol and HDL-C with tamoxifen treatment [135]. Additionally, in experimental studies, tamoxifen exerted effects similar to those of estrogen on vascular relaxation due to several mechanisms, including antagonism of various ion channels [136].

**Expert opinion**

In this work, we tried to provide a literature update on the role of endogenous sex steroids on the cardiovascular system. The sex hormones can act through genomic and non-genomic mechanisms and appear to modulate blood pressure. However, although the activation of ERs is associated with the reduction or attenuation of blood pressure, the action of male sex hormones leads to bidirectional actions on blood pressure that depend on its dose. The use
of testosterone therapy that is beneficial, or at least safe, should be based both on thorough understanding of relationship between endogenous androgens and cardiovascular disease in conditions of health and disease and on findings of controlled trials with relevant end-points, with appropriate design, and of sufficient duration. Unfortunately, experimental evidence for potential beneficial or adverse effects of testosterone on the cardiovascular system is rather limited.

Female sex hormones are able to modulate blood pressure by acting on important systems as cardiovascular, renal, and neural. Female and male sex hormones can have complementary or antagonistic actions. For example, testosterone can raise blood pressure by stimulating the renin-angiotensin-aldosterone system, whereas estrogen alone or combined with progesterone has been associated with decreased blood pressure. Progesterone has both vasodilatory and vasoconstrictive effects in the vasculature depending on location of the vessel and level of exposure. The knowledge of the actions of sex hormones on the cardiovascular system could contribute to the development of more appropriate therapies with sex hormones.

Outlook

The use of sex hormones, especially estrogen, in the cardiovascular system is a complicated clinical issue that requires an in-depth risk/benefit assessment. Much research has been done with the estrogen, but additional research is still needed. After menopause, the development of cardiovascular disease is due not only to estrogen decline but also to testosterone decline. Each of the various clinical trials that have focused on sex hormones has the potential to contribute to our overall understanding but cannot be viewed as the single source of information. This mistake is often made by the public largely, if not solely, due to the media’s sensationalist tendencies. Nonetheless, according to the US Census Bureau, in 2020, 25 million women will be over the age of 65 years. As life expectancy increases, women can expect to live a considerable portion of their lives after menopause. How well they live may depend on how their physicians manage their hormone deficiency. We hope that the results of many experimental studies conducted in vitro and in vivo will be confirmed in prospective studies that will show that sex hormones, when administered properly, exert a protective effect on many systems, including the cardiovascular system. Often, alternative therapies to maintain the well-being after the fall of sex hormones with menopause will improve the quality of life of female users. Finally, we believe more research on G1, a GPER agonist, will advance and show a number of benefits to users without the risk of breast and uterine cancer. The knowledge gained with the study of hormonal therapy can contribute to a better quality of life for women in the future. In addition, it will be necessary to study the involvement of mitochondrial metabolism on cardiac dysfunction observed in estrogen-deficient animals. Future studies involving the improvement of heart function and morbidity and mortality rates of chronic heart failure should focus on the high antioxidant activity.

Highlights

- G1, the agonist of the GPER on the cardiovascular system, lipid profile, oxidative stress, atherosclerosis, and diabetes could represent a new kind of alternative therapy for postmenopausal women without the risk of breast and uterine cancer.
- The effects of SERMs on the cardiovascular system could confirm it as an effective alternative form of therapy in postmenopausal women without the risk of development of breast and uterine cancer.
- Identification of the mechanisms and intracellular pathways that activate GPER may contribute to the development of better forms of therapy.
- New information about testosterone, i.e., whether it has beneficial action on the cardiovascular system, will help physicians and patients when they decide which form of HT they will adopt.
- It is important to identify the mechanisms of rapid (non-genomic) and chronic (genomic) actions of testosterone, which may contribute to the development of better forms of therapy.
- Identification of new forms of alternative therapies will help relieve not only short-term symptoms of menopause but also contribute to the protection of important organ systems such as the cardiovascular, nervous, and renal systems.
- It is necessary to confirm if rapid actions of estrogen are mediated by GPER or by the activation of non-genomic intracellular pathways during the passage of the hormone through the plasmatic membrane.
- Clarifying the responses of sex hormones on the cardiovascular system could provide new information that may contribute to the development of new drugs that can be used in hormonal therapies aimed at enhancing the beneficial actions of sex hormones on the various systems of the human body.
Cardiac contractile dysfunction seems to depend on the increment on myocyte oxidative stress.

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References


35. Mendelsohn ME. Genomic and nongenomic effects of estrogen in the vasculature. Am J Cardiol 2002;90:3F–6F.


TIMES2 Investigators. Testosterone replacement in hypogonadal men with type 2 diabetes and/or metabolic syndrome (the TIMES2 study). Diabetes Care 2011;34:828–37.


